ABSTRACT BOOK OF TRY TAX 2 MEETING OSTRAVA 2016
Trypanosomatidae Biology, Evolution, Taxonomy, and Phylogeny (TryTax2) Meeting

June 14th – 16th
2016
Ostrava
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Foreword

Dear colleagues and friends,

Welcome to the 2nd Trypanosomatidae Biology, Evolution, Taxonomy, and Phylogeny (TryTax2) meeting! The first meeting - TryTax1, organized by Dr. Claudia d'Avila-Levy - took place at FioCruz, Rio de Janeiro, Brazil in April, 2015 and brought together scientists working on many different aspects of these fascinating creatures' biology.

The choice of Ostrava and Czech Republic for the second meeting was not random. First of all, the school of parasitology and protistology in Czech Republic is well established and regarded as one of the best in the world. Secondly, in the last few years, the biological research in Ostrava has greatly advanced putting the city on a "scientific map" along with the centers such as Prague, Brno, and České Budějovice.

The TryTax2, co-organized together with the Czech Society for Parasitology (Česká parazitologická společnost: ČPS), brings under one roof participants from 12 different countries - (in alphabetical order) Australia, Belgium, Brazil, Czech Republic, France, Germany, P.R. China, Russia, Slovakia, Spain, the United Kingdom, and the United States - and will surely stimulate exchange of scientific ideas and will help to conceive new collaborative projects in the field of parasitology and protistology.

Organizing such an event is a big endeavor. This would not be possible without help of our commercial partners (Biotech, KRD, Labmark, Macrogen, Olympus, Sigma-Aldrich, Roche, and VWR) to whom we are grateful. We are also extremely thankful to the Major of the City of Ostrava for the support of our meeting! Last, but definitely not least, we would like to acknowledge the help provided by the University of Ostrava, its Faculty of Science, and the Life Science Research Centre. Our conference would never have happened without truly outstanding assistance of Ing. Jiří Burakowski.

Thanks for your continuous enthusiasm, enjoy the meeting and have fun!

On behalf of the Organizing Committee,

doc. M.Sc. Vyacheslav Yurchenko, Ph.D.

Associate professor and lab head
Laboratory of molecular protozoology
Life Science Research Center
University of Ostrava
Chittussiho 10, 710 00 Ostrava
Czech Republic
vyacheslav.yurchenko@osu.cz
Program Schedule

Tuesday, June 14th

8:00 – 9:00  Registration (Atrium)

9:00 – 9:15  Meeting opening (M-427)
Vyacheslav Yurchenko

Session 1: Evolution (M-427)

9:15 – 10:00  The fascinating evolutionary journey from an excavate ancestor to the first trypanosomatid
Marek Eliáš

10:00 – 10:20  Novel trypanosomatid – bacterium association: a snapshot of an early stage of endosymbiosis evolution
Alexei Kostygov

Kristína Záhonová

10:40 – 11:05  Coffee break

11:05 – 11:50  The flagellum-derived attachment pad and other ancestral features of the kinetoplastid Paratrypanosoma
Julius Lukeš

11:50 – 12:10  Prevalence of RNA viruses in Leishmaniinae
Danyil Grybchuk

12:10 – 12:25  Sponsor's presentation (Olympus): New products for cell culture

12:30 – 13:45  Lunch

Session 2: Trypanosoma spp. (M-427)

13:45 – 14:30  Population genetics, reproductive strategies and taxonomic diversities of African trypanosomes
Thierry De Meeüs

14:30 – 14:50  The ‘Bat Seeding Hypothesis’ and its implications for the evolution of Trypanosoma cruzi
Patrick Hamilton

14:50 – 15:20  Coffee break

15:20 – 15:40  Biology and genomic plasticity in Trypanosoma: Insights from the antioxidant defense and RNAi systems
Edmundo Grisard
15:40 – 16:00 The role of biting midges in avian trypanosomes transmission
Milena Svobodová

16:00 – 16:20 Trypanosoma species diversity infecting bats from Atlantic Forest and Amazonian biomes
Maria Augusta Dario

18:00 – 21:00 Poster Session with wine & cheese

Wednesday, June 15th

Session 3: Leishmania spp. (M-427)

9:15 – 10:00 Evolution and revolution in Leishmania donovani: a phylogenomic approach to drug resistance in the Indian sub-continent
Jean-Claude Dujardin

10:00 – 10:20 Increased prevalence of human cutaneous leishmaniasis in Israel and the Palestine is caused by the recent emergence of genetically similar strains of Leishmania tropica
Gabriele Schönian

10:20 – 10:40 Worldwide population genetic structure of the Leishmania donovani complex based on multi-locus microsatellite and whole-genome SNP variation
Olivia Stark

10:40 – 11:05 Coffee break

11:05 – 11:50 Rat innate resistance to Leishmania amazonensis infection is dependent on the polarized L-arginine metabolism towards the iNOS pathway
Zhao-Rong Lun

11:50 – 12:10 Classical and CRISPR/Cas9-mediated approaches for gene knock-out in Leishmania mexicana
Aygul Ishemgulova

12:10 – 12:25 Sponsor's presentation (KRD): In-Fusion Cloning system

12:25 – 12:30 Group photo

12:30 – 13:45 Lunch

Session 4: Monoxenous Trypanosomatidae (M-427)

13:45 – 14:30 The unexpected diversity of monoxenous trypanosomatids
Jan Votýpka
TryTax2 Meeting Ostrava 2016

14:30 – 14:50  Trypanosomatids taxonomy: from species to typing units
Claudia d'Avila-Levy

14:50 – 15:20  Coffee break

15:20 – 15:40  Isolation and molecular characterisation of a second endemic
Leishmania–like organism in Australia
John Ellis

15:40 – 16:00  Blastocrithidia papi and Leptomonas pyrrhocoris
(Trypanosomatidae) in the true bug Pyrrhocoris apterus
(Pyrrhocoridae): two different life cycle strategies
Anna Ganyukova

16:00 – 16:20  eTryps
Vyacheslav Yurchenko

18:00 – 24:00? Gala dinner

Thursday, June 16th

Session 5: Metabolism. (M-427)

9:15 – 10:00  Endocytosis in trypanosomes: Drugs and evolution
Mark Field

10:00 – 10:20  Mitochondrial gene expression is responsive to starvation stress
and developmental transition in Trypanosoma cruzi
Sara Zimmer

10:20 – 10:40  FtsH protease of Trypanosoma brucei
Anton Horváth

10:40 – 11:05  Coffee break

11:05 – 11:50  Genome wide localization of RNA polymerase II by ChIP-seq
analysis identifies functional promoter sequences in African
trypanosomes
Miguel Navarro

11:50 – 12:10  Queuosine: The role of an essential tRNA modification in
parasitic protist Trypanosoma brucei
Zdeněk Paris

12:10 – 12:30  Development of a toolbox to dissect host-endosymbiont
interactions and protein trafficking in the trypanosomatid
Angomonas deanei
Jorge Morales
12:30 – 13:45  Lunch

Session 6: Genomics (M-427)

13:45 – 14:30  Trypanosomatidae genome evolution gets ambiguous
Joao Alves

14:30 – 14:50  A high quality genome of Leptomonas pyrrhocoris sheds light on
evolution of parasitism in trypanosomatids
Pavel Flegontov

14:50 – 15:20  Coffee break

15:20 – 15:40  T-Aligner: RNA-editing reconstruction and visualization tool
Evgeny Gerasimov

15:40 – 16:00  Best poster award and closing remarks

17:00 – 20:00  Dolní oblast Vítkovice - The National Culture Monument trip
Lectures
Trypanosomatidae genome evolution gets ambiguous

Alves, J.M.P.\textsuperscript{1}, Morais, A.C.\textsuperscript{1}, Serrano, M.G.\textsuperscript{2}, Buck, G.A.\textsuperscript{2}, Teixeira, M.M.G.\textsuperscript{1}, Camargo, E.P.\textsuperscript{1}

\textsuperscript{1}Dept. of Parasitology, Institute of Biomedical Sciences, University of São Paulo, Brazil
\textsuperscript{2}Dept. of Microbiology and Immunology, Virginia Commonwealth University, USA

Due to their great medical significance, organisms from the Trypanosomatidae family were relatively early participants in the genomic revolution, with the genome sequences of \textit{Trypanosoma cruzi}, \textit{T. brucei}, and \textit{Leishmania major} first coming out more than a decade ago already, in 2005. On the other hand, genomes from other trypanosomatid organisms outside these two genera have not received much attention until very recently, when dramatic drops in cost and time for whole genome sequencing took place.

Examples of the recent progress in genomic studies of Trypanosomatidae are the publications involving monoxenous insect-infecting trypanosomatids such as \textit{Strigomonas}, \textit{Angomonas}, \textit{Lotmaria}, and \textit{Leptomonas}, which are uncovering many interesting aspects of the biology and evolution of these organisms, specially at the genetic level. One specially interesting insect-infecting trypanosomatid is \textit{Angomonas ambiguus}, whose name already points to its convoluted evolutionary history. Like other symbiont-harboring trypanosomatids (SHT), \textit{A. ambiguus} contains a single bacterial symbiont (TPE, for Trypanosomatid Proteobacterial Endosymbiont) living inside the eukaryotic cell, in an ancient co-evolving partnership. In most of the SHT species, there is strictly vertical inheritance of the TPE by new SHT generations, but that has been previously reported not to be the case in \textit{A. ambiguus}: this trypanosomatid's TPE seems to belong to the same species as (or something very closely related to) the TPE from \textit{Angomonas deanei}, while the nuclear genome indicates closer phylogenetic relationship to the \textit{Angomonas desouzai} species.

In this work, we present the preliminary studies on the evolution of the \textit{A. ambiguus} genome, analyzing both the TPE and SHT genomes, in comparison with the five previously available SHT/TPE genome pairs, in order to better understand the phylogenetic origins of the \textit{A. ambiguus} genomes, with the expectation of refining our understanding of endosymbiosis evolution in these organisms.
The diversity of monoxenous trypanosomatids that are found in the digestive tract or salivary glands of insects has been for a long time overlooked. It is interesting to note that insects usually top the count of species diversity, while if we examine the contents of a single insect, we will find hundreds or thousands of distinct microbial species, which makes us feel that we are only seeing the tip of the iceberg. One possible reason is that few research groups devoted their time, knowledge and efforts to unveil this protozoa group diversity, occurrence and distribution. If we decide to scrutiny the Kinetoplastea class, which has several free-living protozoa, the knowledge is even more fragmented. This scenario is reflected in the paucity of catalogued information on trypanosomatids diversity and taxonomic classification. Very simple questions remain unanswered or partially answered, such as, (i) what is the total number of described species? (ii) what are the objective criteria to distinguish trypanosomatid species? (iii) what is the number of species available for the scientific community in culture collections? Therefore, the work of a taxonomist and collector of trypanosomatids is even more puzzling and hard. The correlation of a new isolate to previously described ones is not a simple task, mainly due to this scenario and not due to difficulties in methodological approaches. The FIOCRUZ Protozoa Culture Collection has about 300 Kinetoplastea isolates, and molecular typing of these isolates indicates that sometimes two, three or even more described and validated species represent one single molecular typing unit, which is not surprising if we consider what was exposed above, together with the principles of trypanosomatids taxonomy used for a long time: host specificity and morphological characteristics, which is nowadays clear that does not necessarily provide a correlation with molecular relationship of the isolates. Therefore, in the internet era, with a high speed data sharing, it is time for taxonomist working on trypanosomatids to feed up online databases with sequences, images and sample collection information. Also, an international guideline for species description and validation should be compiled. One key point to be addressed should be the obligation to deposit the new described isolates in Institutional Culture Collections, such as those affiliated to World Federation for Culture Collections (WFCC), to warrant preservation of biological resources, data management and accessibility of the species to the scientific community.
Trypanosoma species diversity infecting bats from Atlantic Forest and Amazonian biomes

Dario, M.A.¹, dos Santos, F.C.B², Lisboa, C.V.¹, Moratelli, R.³, de Sousa Verde, R.⁴, Calouro, A.M.⁴, Roque, A.L.R.¹, Jansen, A.M.¹

¹Laboratório de Biologia de Tripanosomatídeos, IOC/Fiocruz, Rio de Janeiro/RJ, Brazil
²Instituto Federal do Acre, Rio Branco/AC, Brazil
³Campus Fiocruz Mata Atlântica, CFMA/Fiocruz, Rio de Janeiro/RJ, Brazil
⁴Laboratório de Ecologia de Mamíferos, Rio Branco/AC, Brazil

Background. Trypanosoma species from the Schizotrypanum subgenus are parasites exclusively found in bats, except for Trypanosoma cruzi, which also infects other mammals. Another parasite widely distributed and that can be found infecting bats is T. rangeli. Bats are worldwide distributed, and comprise more than 1200 species. The role of bats in the dispersal of these parasites in nature is still little understood. The aim of this study was to survey Trypanosoma species infecting bats in two Brazilian biomes: Amazonian and Atlantic Forest.

Methods: Fieldwork was conducted in the states of Acre (AC) and Espírito Santo (ES), representing Amazon and Atlantic Forest biomes. Bats were sampled during three expeditions, using 4–8 mist nets per sampling, for two nights in three (ES) or six (AC) locations. DNAs extracted from positive hemocultures were submitted to PCR amplification of the SSU rRNA and gGAPDH genes, followed by purification and DNA sequencing. The sequences obtained were edited and phylogenetic trees were constructed, using maximum likelihood method, with 1000 bootstrap.

Results/Conclusions. Ten species from 129 specimens and 24 species from 281 specimens were examined in Atlantic Forest and Amazon biomes. T. dionisii prevailed in the Atlantic Forest (n = 10), while only one Carollia sp. bat was infected by this species in the Amazon. T. c. marinkellei and T. cruzi (DTU TcI) were the main species found in the Amazon (n= 6). No T. cruzi infection was detected in bats from the Atlantic Forest. Phyllostomus sp. bats were infected by T. c. marinkellei in both biomes, confirming again that T. c. marinkellei is not restricted to the Brazilian northern and central regions. T. rangeli genotype D, up to now only reported infecting rodents, was found infecting a bat (Carollia sp.) in the Atlantic Forest. Bats from both biomes harbor high diversity of Trypanosoma species and, at least in Amazon, they may be involved in the maintenance of T. cruzi in Chagas disease endemic areas.
Population genetics, reproductive strategies and taxonomic diversities of African trypanosomes

De Meeûs, T.¹, Koffi, M.², Séré, M.³, Weir, W.⁴

1. Institut de Recherche pour le Développement, Unité Mixte de Recherche IRD-CIRAD 177, Campus International de Baillarguet, 34398 Montpellier, Cedex 5, France
2. Université Jean Lorougnon GUEDE, UFR Environnement, Laboratoire des Interactions Hôte-Microorganismes-Environnement et Evolution (LIHME), BP 150 Daloa, Côte d'Ivoire
3. Centre International de Recherche-Développement de l’Elevage en zone Subhumide (CIRDES), 01 BP 454 Bobo-Dioulasso 01, Burkina Faso
4. Wellcome Centre for Molecular Parasitology, College of Medical, Veterinary and Life Sciences, Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow, Henry Wellcome Building, Garscube Campus, Bearsden Road, Glasgow, G61 1QH, UK

There are still many issues regarding the taxonomy, ecology and reproductive modes of African trypanosomes. This is not only a matter of pure science because knowing more precisely the ecological diversity and reproductive modes of these pests can prove useful for understanding parasite adaptation, as well as how parasitism, specialization (parasite specificity), and complex life cycles evolve over time. This can also provide key information to the understanding of the epidemiology of associated diseases and clues for elaborating control programs and predicting the probability of success for control campaigns (such as vaccines and drug therapies), along with emergence or re-emergence risks. Population genetics tools, if appropriately used, can provide precise and useful information in these investigations. In this paper, we revisit published data collected during population genetics surveys of different Trypanosoma species in sub-Saharan Africa. We use these to discuss on issues regarding future directions of research, especially so vis-à-vis particular lineages of Trypanosoma brucei species complex and its odd taxonomy.
Evolution and revolution in *Leishmania donovani*: a phylogenomic approach to drug resistance in the Indian sub-continent

*Dujardin, J.C.¹, Imamura, H.¹, Van den Broeck, F.¹, Downing, T.², Domagalska, T.¹, Dumetz, F.¹, Rai, K.³, Rijal, S.³, Sundar, S.⁴, Sanders, M.⁵, Berriman, M.⁵, Cotton, J.⁵*

¹Institute of Tropical Medicine, Antwerp, Belgium
²NUI Galway, Ireland
³BPKIHS, Dharan, Nepal
⁴Banaras Hindu University, Varanasi, India
⁵Wellcome Trust Sanger Institute, Hinxton, UK

In order to study the evolution of *Leishmania donovani* in the Indian subcontinent, we sequenced the entire genomes of 204 clinical isolates (all at promastigote stage) from India, Nepal and Bangladesh. We found two genetically distinct lineages: (i) the main population (‘Core-191’) was very homogeneous (2,418 SNPs), was endemic in the Gangetic lowlands and likely emerged around 1850 (predicted by BEAST), corresponding to the first historical reports on visceral leishmaniasis (VL) outbreak in the region; (ii) the ‘Yeti-12’ was also homogeneous (5,628 SNPs) and originated from hilly districts in Nepal. The core-191 was split in 6 common populations, 13 rare types, and 8 isolates showing signs of recombination. The most significant population bottleneck in the history of the core-191 occurred in 1960s during the antimalarial DDT campaign: after that, the *L. donovani* populations expanded to produce the recent epidemics. Our study highlights 3 events leading to the emergence of drug resistance: (i) the radiation of SSG-resistant strains in the ‘60s followed by geographic separation and formation of a SSG-resistant clade (ISC5), (ii) the more recent and independent multiple emergence of SSG-resistant strains within other ‘SSG-sensitive’ populations (ISC2-7), and (iii) sexual recombination of ISC5/6/7. Genes involved are not necessarily the same in the 3 cases. Contrasting with the low SNP diversity expected in a young population, we observed a dramatic level of variation in gene dosage, both locally and at whole chromosome level: 33 out of 36 chromosomes showed a variable somy in 80% of the isolates. Furthermore, we observed different degrees of aneuploidy in the same isolate from hamster amastigotes compared to sand flies promastigotes. This suggests the hypothesis that chromosomal copy number patterns in *L. donovani* could be stage- and/or environment-specific. Accordingly, it is essential in the next step to develop sequencing methods allowing a direct sequencing of clinical samples, without isolating the parasites. We will present our latest advances in this endeavor.
The fascinating evolutionary journey from an excavate ancestor to the first trypanosomatid

Eliáš, M.

Life Science Research Centre; Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10, Ostrava, Czech Republic

Although often claimed to be “ancient”, “primitive”, or “deeply branching” protists, trypanosomatids are not either of those. In my talk I will provide an up-to-date perspective on the position of trypanosomatids in the eukaryote phylogeny and on the evolutionary origin of the various trypanosomatid traits that make them so special organisms. I will discuss the recent evidence for a particular hypothesis on the position of the root of the eukaryote phylogeny that implies that the last eukaryotic common ancestor (LECA) was a small bacteriovorous excavate flagellate similar to jakobids and malawimonads. I will then follow the evolutionary path from this hypothetical ancestor ultimately leading to trypanosomatids and will comment on the most important evolutionary events that gradually transformed the ancestral eukaryote into the first trypanosomatid. In my evolutionary narrative I will not only reflect upon published data, but will also incorporate some relevant unpublished results reached in my laboratory. The central take-home message of my talk should be that the presumed primitiveness or ancient nature of trypanosomatids (or euglenozoans as a whole) is a myth. Quite the opposite, in evolutionary terms trypanosomatids and their relatives are one of the most advanced (or derived) eukaryote groups that have abandoned many ancestral and widespread eukaryotic traits and have instead evolved their own peculiar life's solutions.
Isolation and molecular characterisation of a second endemic Leishmania–like organism in Australia

Kaufer, A.1, Ellis, J.1, Stark, D.2, Barratt, J.1

1School of Life Sciences and the i3 Institute, University of Technology Sydney, PO Box 123, Broadway, NSW 2007, Australia
2Department of Microbiology, St. Vincent’s Hospital, Darlinghurst, NSW 2010, Australia

Australia has long been considered a continent free of endemic of Leishmania spp., however the increasing incidence of imported cases of leishmaniasis due to international travellers, immigrants and military personnel is alarming. Consequently biosecurity and surveillance is paramount to preventing the establishment of Leishmania in this country. The discovery of a native Kangaroo-infecting Leishmania parasite (2004) and its day feeding, midge vector Forcipomyia (2011) shows that such parasites can exist. As part of a recent study, a second Leishmania-like parasite was isolated from a blood-feeding insect in Australia’s Northern Territory. Detailed morphological and molecular analyses were performed to aid in this organisms’ classification. The organism was initially maintained xenically in vitro using M199 medium containing heat inactivated horse serum (and pen/strep). Axenic cultures were established by plating organisms onto chocolate agar plates and reinoculating colonies into liquid M199 media. Light and electron microscopy performed on flagellates from a pure culture confirmed that this organism is morphologically indistinguishable from Leishmania and Leptomonas spp. Phylogenetic analyses, using four genes (SSU-rDNA, RPOIILS, Hsp70 and GAPDH) confirmed it was basal to all Leishmania and formed a sister group with an organism previously described as Leptomonas costaricensis. Based on these detailed morphological and molecular analyses, we are unable to confidently assign this taxon to a genus without further information about its biology. Future studies will investigate the host distribution of this flagellate and the nature of its development in a host.
Endocytosis in trypanosomes: Drugs and evolution

Field, M.C., Manna, P., Leung, K., Zoltner, M., Horn, D.

School of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK

Intracellular transport is a major aspect of eukaryotic cell physiology, and has its origins with the earliest eukaryotes. The system has been adapted in many lineages and this is presumed to be a feature of the lifestyle and specific ecological niche that each organism occupies. Parasitic trypanosomes have an especial burden, in terms of avoiding the immune response of their hosts. In the African trypanosome there are clear modifications to the basic endocytic machinery. Here I will discuss several of these findings, together with the identification and characterisation of novel proteins involved in endocytosis and which appear trypanosome specific. Further, recent evidence indicates an intimate connection between the parasite endocytic apparatus and sensitivity and uptake to drugs currently in the clinic, suggesting that this system may offer a means to target the parasite therapeutically.
A high quality genome of *Leptomonas pyrrhocoris* sheds light on evolution of parasitism in trypanosomatids

*Flegontov, P.¹,²,³, Butenko, A.², Kraeva, N.², Field, M.C.⁵, Filatov, D.⁶, Ishemgulova, A.², Yurchenko, V.¹,²,⁴, Lukeš, J.¹,⁷,⁸*

¹Biology Centre, Institute of Parasitology, Czech Academy of Sciences, 370 05 České Budějovice (Budweis)
²Life Science Research Centre, Faculty of Science, University of Ostrava, 710 00 Ostrava
³Institute for Information Transmission Problems, Russian Academy of Sciences, 127051, Moscow, Russia
⁴Institute of Environmental Technologies, Faculty of Science, University of Ostrava, 710 00 Ostrava
⁵School of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK
⁶Department of Plant Sciences, University of Oxford, Oxford, OX1 3RB, UK
⁷Faculty of Science, University of South Bohemia, 370 05 České Budějovice (Budweis)
⁸Canadian Institute for Advanced Research, Toronto, ON M5G 1Z8, Canada

The trypanosomatids (Trypanosomatidae, Kinetoplastea) are a group of exclusively parasitic protists that can have a dixenous life cycle involving a secondary host (either a vertebrate or a vascular plant) or a simpler monoxenous (one-host) life cycle confined to insects. Many high-quality genomes are available for dixenous trypanosomatid species of the genera *Trypanosoma*, *Leishmania*, and *Phytomonas*, but only fragmentary information is available for monoxenous species. In trypanosomatids, monoxeny is ancestral to dixeny, thus it is anticipated that the genome sequences of the key monoxenous parasites will be instrumental for both understanding the origin of parasitism and the evolution of dixeny. We have sequenced and assembled the genome of monoxenous trypanosomatid, *Leptomonas pyrrhocoris*, which is closely related to the dixenous genus *Leishmania*. The *L. pyrrhocoris* genome (30.4 Mbp in 60 scaffolds) encodes 10,148 genes. Using the *L. pyrrhocoris* genome, we pinpointed genes gained in *Leishmania*. Among those genes, 20 genes with unknown function had expression patterns in the *Leishmania mexicana* life cycle suggesting their potential role in virulence. By combining differential expression data for *L. mexicana*, *L. major* and *Leptomonas seymouri* with phyletic patterns, we have identified several additional proteins potentially involved in virulence, including SpoU methylase and U3 small nucleolar ribonucleoprotein IMP3. The population genetics of *L. pyrrhocoris* was also addressed by sequencing thirteen strains of different geographic origin, allowing the identification of 1,318 genes under positive selection. GO terms enrichment analysis revealed that this set of genes was significantly enriched in components of the cytoskeleton and the flagellum.
**Blastocrithidia papi** and **Leptomonas pyrrhocoris** (Trypanosomatidae) in the true bug **Pyrrhocoris apterus** (Pyrrhocoridae): two different life cycle strategies

*Ganyukova, A.I., Malysheva, M.N., Kostygov, A.Y., Frolov, A.O.*

Protozoology lab., Zoological Institute of the Russian Academy of Sciences, St. Petersburg, 199034, Russia

We investigated the two-component trypanosomatid community consisting of *Blastocrithidia papi* and *Leptomonas pyrrhocoris* which parasitize the true bug *Pyrrhocoris apterus*. This study was conducted on insects collected from a single colony on the north of Pskov Region (Russian Federation). Phylogenetic analysis using sequences of 18S rRNA and SL genes revealed only two species of monoxenous trypanosomatids: *B. papi* and *L. pyrrhocoris*. Both parasites' life cycles include long-term persistence in the host due to winter diapause lasting up to six months as well as active proliferation and dispersal. The following laboratory cultures were obtained during this study: two cultures of both trypanosomatid species and four host cultures – one naturally-infected, one uninfected and two experimentally infected (with *B. papi* and *L. pyrrhocoris* respectively). We demonstrated that both species of parasites localize mainly in the host's midgut regardless of whether the infection is mixed or not. *L. pyrrhocoris* prefers the anterior part of the midgut (M1 and M2) and *B. papi* occupies the sacciform region (M3). Furthermore, *L. pyrrhocoris* is able to penetrate into the body cavity (haemocoel) and infect endothelial system cells, salivary glands and haemolymph. *B. papi* forms flagellar “cysts” inside the host's Malpighian tubules. We characterized ultrastructural organization of both trypanosomatid species and their interaction with various cells and tissues of the insect host. A series of experiments allowed us to determine several transmission routes used by the two species of trypanosomatids. Horizontal transmission is mainly realized either through necrophagy or through a contaminated substrate. In *B. papi* we detected vertical transmission by contamination of the eggs’ surface with “cysts”. We discuss the life cycle strategies of both trypanosomatid species, host-parasite relationships and interspecific interactions between two co-infecting flagellates.
T-Aligner: RNA-editing reconstruction and visualization tool

Gerasimov, E.S.¹, Gasparyan, A.V.¹, Kaurov, I.², Logacheva, M.D.³, Kolesnikov, A.A.¹, Flegontov, P.N.⁴

¹Department of Biology, M.V. Lomonosov Moscow State University, 119991, Moscow, Russia
²Biology Centre, Institute of Parasitology, Czech Academy of Sciences, 370 05 České Budějovice, Czech Republic
³Belozersky Institute of Physico-Chemical Biology, M.V. Lomonosov Moscow State University, 119991, Moscow, Russia
⁴Department of Biology and Ecology, University of Ostrava, Ostrava, 710 00, Czech Republic

Cryptogenes are ORF-less loci of kinetoplast maxicircle which expression is mediated by a complex process of U-insertions/deletions in their transcripts. This process is called RNA editing and is guided by special short RNA molecules (guide RNAs) encoded by minicircles. Thus, RNA editing assembles information from genetically distinct genome loci and combines it into functional mRNA.

Edited mRNA’s sequence and cryptogene’s sequence are usually too dissimilar to be aligned with general-purpose sequencing read aligners (like Bowtie2). To study RNA editing using NGS technology we developed our own tool T-Aligner, which is capable to align RNA edited reads against cryptogene reference and draw editing pathway graphs. We show that our algorithm is much more sensitive than Bowtie2, even as compared to its modified version tailored to U-indel-edited reads (previously published by us).

We use T-Aligner to build editing graphs for maxicircle transcripts of monoxenous trypanosomatid Leptomonas pyrrhocoris H10 and show that RNA editing can produce rather complex and divergent sets of sequences from single cryptogene. Also some cryptogene transcripts are targets of so-called alternative editing producing two radically different ORFs.
Biology and genomic plasticity in *Trypanosoma*: Insights from the antioxidant defense and RNAi systems

**Grisard, E.C., Stoco, P.H.**

Universidade Federal de Santa Catarina, Brazil

The sequencing of the TriTryps genomes (*T. brucei, T. cruzi* and *Leishmania major*) has unveiled the genomic plasticity of the pathogenic trypanosomatids. More recently, genomes from other pathogenic, non-pathogenic and monoxenous trypanosomatids allowed comparative phylogenomic studies, pointing out a major diversity and some conserved core genes. In this sense, we have addressed the evolution of the antioxidant and RNAi systems of trypanosomatids at the genomic level, proposing hypothesis on their possible influence on these organisms’ genome structure and evolution. The existence of a functional RNAi system among trypanosomatids was firstly described in *T. brucei* and then in *L. braziliensis*, being non-functional or not described to any other trypanosomatid species. As a cellular mechanism to control gene expression, the RNAi machinery in *T. brucei* is basically composed of five genes (DCL1, DCL2, AGO, RIF4 and RIF5) and acts on degradation of both endogenous and exogenous dsRNA. *T. rangeli*, a non-pathogenic hemoflagellate protozoan parasite infecting humans, wild and domestic mammals in Central and South America, has the shortest haploid genome among the sequenced trypanosomes (~27.7Mb), being less variable if compared to *T. cruzi* and *T. brucei*. This parasite also presents intraspecific karyotype variability and a remarkable reduction on the number of gene copies on the most important *T. cruzi* multigene families (Sialidases/Trans-Sialidases, Mucins and MASP5). The *T. rangeli* genome contains a complete DCL2 gene and truncated orthologs (pseudogenes) corresponding to the other RNAi genes from *T. brucei* (DCL1, AGO, RIF4 and RIF5), but the machinery proved to be non-functional. Interestingly, the complete DCL2 gene is exclusively present on *T. rangeli* strains from the KP1+ lineage, being a pseudogene on KP1- parasites, among which, the AGO and DCL1 pseudogenes revealed increased size and sequence variability if compared to the KP1+ parasites. The shorter genome size and the apparently loss of a functional RNAi machinery in *T. rangeli* contrasts with the complete absence of orthologs of the RNAi machinery genes and the
expansion and plasticity of the genome size in *T. cruzi* and to the genome organization and the presence of an active RNAi system in *T. brucei*. The same is observed between the *L. braziliensis* (RNAi +) and *L. amazonensis* (RNAi -) genome sizes. We hypothesized that loss/maintenance/gain of RNAi machinery may have influenced on the size and sequence variability of the trypanosomatids genomes, although the presence of mi/ siRNA in species that does not have a classic RNAi machinery as described for *T. brucei* may indicate other mechanisms of dsRNA degradation. Targeting the antioxidant system, we have performed comparative *in silico* analyses of the enzymes involved on response to oxidative (ROS) and nitrosative (RNS) stresses to propose a model for the evolution of this system among the distinct trypanosomatid species. All analysed genes were identified in the free-living ancestral protozoa *Bodo saltans*, suggesting that existence of antioxidant defence mechanisms have evolved before the adaptation of the Trypanosomatid clade. In this sense, most of monoxenous parasites and all *Leishmania* species had only few differences in the number of gene copies if compared to *B. saltans*. However, the endosymbiont-bearing *Angomonas deanei* and *Strigomonas culicis* showed increased copy number to the majority of the genes related to ROS / RNS. Also, absences of some genes related to ROS/RNS were especially observed in *Phytomonas* spp. and *Trypanosoma* spp. The lack of CS, POT1, GspS and APx appears to be common among several species with extracellular parasitic lifestyle like *Phytomonas* sp., *T. brucei*, *T. vivax* and *T. evansi*. An exception to this observation was *T. grayi*, an extracellular parasite of crocodiles transmitted via feces of Tsetse flies. Comparative analyses of the antioxidant system allowed a clear distinction between stercorarian and salivarian trypanosome species. The analysis of the presence/absence of ROS/RNS-related genes placed *T. cruzi* closely related to the African *T. grayi* than to the South American *T. rangeli*. This seems to indicate that the presence and evolution of specific antioxidant defence pathways are related to each species adaptation to parasitism instead of a result of a geographical isolation.
Prevalence of RNA viruses in Leishmaniinae

Grybchuk, D.1, Kostygov, A.1, Lukeš, J.2, Yurchenko, V.1,2

1Life Science Research Centre, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czech Republic
2Biology Centre, Institute of Parasitology, Czech Academy of Sciences, 370 05 České Budejovice (Budweis), Czech Republic

We conducted a broad screening of RNA viruses in 46 monoxenous representatives of Leishmaniinae subfamily. Viruses were found in eight individual isolates: Crithidia otxongatchiensis, Crithidia sp. ZM, Crithidia sp. C4, Trypanosomatidae sp. G15, Leptomonas seymouri, Leptomonas pyrrhocoris (isolates F19, F165 and H10).

Currently, three types of viral RNA-dependent RNA polymerases (RDRPs) were detected. All Crithidia species harbored a single 6 kb long viral RNA coding for Phlebovirus-like RDRP. Phleboviruses are negative strand RNA viruses transmitted by sand flies. L. seymouri virus possessed a RDRP closely related to Narnavirus, which are described from various fungi and oomycetes, but it also contained an additional RNA segment not present in previously studied species. RDRP and overall genomic structure of the L. pyrrhocoris virus were reminiscent of the unclassified insect viruses infecting bees (Chronic bee paralysis virus) and Drosophila (Dansoman virus). A closely related RDRP sequence was also found integrated into L. pyrrhocoris genome.

Presence of insect-specific viruses has never been documented in protists before. Thus, monoxenous trypanosomatids can be view as a novel model for studying the ecology and biology of RNA viruses. These findings suggest a possible exchange of viruses between trypanosomatids and their insect hosts during their shared evolutionary history, although these taxa are related quite distantly. In addition, a stable viral infection in L. seymouri together with the absence of RNAi machinery in this species implies a selection-driven retention of the virus. Previously, this phenomenon was observed only in Leishmania species capable of infecting mammals, pointing to the possibility that L. seymouri is preadapted for dixenous lifecycle.
The ‘Bat Seeding Hypothesis’ and its implications for the evolution of

*Trypanosoma cruzi*

*Hamilton, P.*¹, *Teixeira, M.M.G.*², *Stevens, J.*¹

¹School of Biosciences, University of Exeter, Exeter, Devon EX4 4PS, UK
²Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP 05508-900, Brazil

Until recently, discussion of the evolution of *Trypanosoma cruzi* has been dominated by the southern super-continent hypothesis, whereby *T. cruzi* and related parasites (the *T. cruzi* clade) evolved in isolation in the mammals of South America, Antarctica and Australia. This hypothesis has been central in attempts to date the origin of *T. cruzi* and has been used for dating divergence of trypanosomes more generally. Since its proposal, knowledge of trypanosome diversity in wildlife has advanced considerably. Increased knowledge of the composition of the *T. cruzi* clade led to an alternate scenario, the ‘bat seeding hypothesis’ which suggests that *T. cruzi* evolved from within a broader clade of bat trypanosomes, and that bat trypanosomes have successfully made the switch into other mammalian hosts in both the New and Old Worlds. A series of studies that have characterised bat trypanosomes using sequencing of genes for 18S rRNA and *gGAPDH* and fluorescent fragment length barcoding (FFLB) provided key evidence in support of the hypothesis. The recently-described bat trypanosomes all fall within the *T. cruzi* clade yet are diverse, being distantly related to each other within the group. There have been several transitions from bat to terrestrial hosts within the clade, including in the Old World as the clade is now known from primates in Africa and Madagascar. Additional evidence in support of the new hypothesis has been derived from studies of trypanosomes in Australian mammals that show there are at least five evolutionary groups in mammals in the continent, demonstrating that geographical isolation is not a major barrier to the spread of trypanosomes over evolutionary time. Furthermore, studies have also shown very closely related strains of bat trypanosome in Brazil and Europe, demonstrating that trypanosomes of bats can spread between isolated continents. As the *T. cruzi* clade is unlikely to predate the origin of bats, this provides an opportunity to date divergence within the clade.
FtsH protease of *Trypanosoma brucei*

*Kováčová, B., Kovalinka, T., Horváth A.*

Department of Biochemistry, Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovakia

Protozoan parasite *Trypanosoma brucei* (Euglenozoa, Kinetoplastea, Trypanosomatida) is the causative agent of sleeping sickness in human and disease Nagano in animals. Better cognition of this organism is necessary for successful fight against caused diseases. In order to study *T. brucei* mitochondrial metabolism we have focused on mitochondrial FtsH protease that was not closely studied among the trypanosomatids so far. FtsH protease is a representative of mitochondrial AAA proteases associated with various cell activities. Functional enzyme is composed of six subunits. In yeast *S. cerevisiae* and in human were identified three homolog subunits of FtsH. They can form either homo- or hetero-hexameric structures oriented either to matrix or intermembrane space. Orientation is determined by number of transmembrane domains on N terminus of subunits. *T. brucei* possess six FtsH homologs. We have prepared cell lines for inducible RNAi of each subunit. Influence of gene silencing on growth phenotype and activity of oxidative phosphorylation enzymes were studied. We have also performed in silico analysis for the presence of trans-membrane domains of these six genes.
Classical and CRISPR/Cas9-mediated approaches for gene knock-out in 

*Leishmania mexicana*

*Ishemgulova, A.¹, Flegontov, P.¹,², Hlaváčová, J.³, Votýpka, J.³, Lukeš, J.²,⁴, Yurchenko, J.²,⁴*

¹University of Ostrava, Faculty of Science, Life Science Research Centre, Ostrava  
²ASCR, Institute of Parasitology, České Budějovice  
³Charles University in Prague, Faculty of Science, Department of Parasitology, Praha  
⁴University of South Bohemia, Faculty of Science, České Budějovice

*Leishmania* are protozoan parasites from the family Trypanosomatidae causing leishmaniasis. This disease represents a major public health risk in many tropical and subtropical regions of the world. There is no satisfactory treatment against leishmaniasis, thus understanding biology of this parasite and investigation of its virulence factors are important. We have identified several novel putative virulence factors in *Leishmania* infection and generated several knock-out lines ablating these genes to test their involvement in *Leishmania* pathogenicity.

Classical knock-out strategies are based on replacement of a target gene by the antibiotic resistance cassette through homological recombination. *Leishmania* is aneuploid organism which causes difficulties in targeted genome modification using classical strategies. The CRISPR/Cas9-mediated knock-out strategy allows overcoming this hurdle. In this work we compared advantages and disadvantages of classical and CRISPR/Cas9-mediated approaches for targeted genome modification in *Leishmania mexicana.*
As a result of a broad-scale survey of insect trypanosomatid biodiversity in Ecuador we discovered a novel symbiotic association between a kinetoplastid protist *Novymonas esmeraldas* and an intracytoplasmic bacterium *Ca. Pandoraea novymonadis*. We characterized this association by describing morphology of both organisms, as well as their interactions, and by establishing their phylogenetic affinities. Importantly, neither partner was found to be closely related to other known organisms previously implicated in eukaryote-bacterial symbiosis. This symbiotic association seems to be relatively recent, as the host does not exert a stringent control over the number of bacteria harbored in its cytoplasm. We argue that this unique relationship may represent a suitable model for studying the initial stages of establishment of endosymbiosis between a single-cellular eukaryote and a prokaryote. Based on phylogenetic analyses, *Novymonas* could be considered a proxy for the insect-only ancestor of the dixenous genus *Leishmania*, and shed light on the origin of the two-host life cycle within the subfamily Leishmaniinae.
The flagellum-derived attachment pad and other ancestral features of the kinetoplastid *Paratrypanosoma*

*Skalický, T.¹,², Dobáková, E.¹, Tesařová, M.¹, Flegontov, P.¹,³, Jirsová, D.¹, Votýpka, J.¹,⁴, Yurchenko, J.¹,³,⁵, Ayala, F.⁶, Lukeš, J.¹,²,⁷*

¹Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Budweis), Czech Republic
²Faculty of Science, University of South Bohemia, České Budějovice (Budweis), Czech Republic
³Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czech Republic
⁴Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic
⁵Institute of Environmental Technologies, Faculty of Science, University of Ostrava, Ostrava, Czech Republic
⁶Department of Ecology and Evolutionary Biology, University of California, Irvine, USA
⁷Canadian Institute for Advanced Research, Toronto, Canada

*Paratrypanosoma confusum* is a kinetoplastid flagellate that constitutes earliest known branch of the diverse obligatory parasitic trypanosomatid clade and is hence the closest relative of the free-living *Bodo*. Its genome has already been streamlined and reduced as compared to *Bodo*, and is quite similar to the genomes of monoxenous trypanosomatids. However, two documented life stages have very different transcriptomes, reflecting their strikingly distinct morphology. While the swimmer stage is a typical promastigote, the other stage divides when attached to the surface via an extensive flagellum-derived attachment pad. This sessilemastigote morphotype does not fell into the liberform and juxtaform superclasses and represents a category of its own. The promastigote stage already has several hallmarks of the highly successful trypanosomatids, yet the morphology and the absence of social motility of the sessilemastigote reveal that the monoxenous *P. confusum* still retains ancestral features lost by all derived lineages.
Rat innate resistance to *Leishmania amazonensis* infection is dependent on the polarized L-arginine metabolism towards the iNOS pathway.


1Center for Parasitic Organisms, Key laboratory of Gene Engineering of the Ministry of Education, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, PR China
2Institute of Immunology and Key Laboratory of Tropical Disease Control of the Ministry of Education, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou 510080, P. R. China
3Ecosystems and Environment Research Centre and Biomedical Research Centre, School of Environment and Life Sciences, University of Salford, UK
4Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA

*Leishmania* infection causes diverse clinical manifestations in human, and the disease outcome is complicated by the combination of many factor from host and parasite. Inbred mouse strains are vary in resistance to *L. major* but are highly susceptible to *L. amazonensis* infection. However, most rat strains are highly resistance to *L. amazonensis* infection, while mechanism remains unknown. In this study, by using the inducible nitric oxide synthase (iNOS) gene knockout rodent model, we identified L-arginine metabolism of rat macrophages which predominantly diverted towards iNOS pathway as the key factor for rat resistance against *L. amazonensis* infection. Accordingly, iNOS deficient rats are susceptible to *L. amazonensis* infection even though the immune response is unimpaired. Moreover, adoptive transfer of iNOS competent macrophage to KO rats significantly reduced the disease development. Thus, differential L-arginine metabolism in rat macrophage is the basis for the strong innate resistance to *Leishmania*, highlighting that macrophages from different host possess distinctive properties and functions in the innate immunity during *Leishmania* infection. (Work was supported by grants (#31272305 and #31472058) from the National Natural Science Foundation of China to ZRL).
Development of a toolbox to dissect host-endosymbiont interactions and protein trafficking in the trypanosomatid *Angomonas deanei*

*Morales, J.*, *Kokkori, S.*, *Nowack, E.*

Department of Biology, Heinrich-Heine-Universität Düsseldorf, 40255 Düsseldorf, Germany

Endosymbiotic bacteria gave rise to mitochondria and plastids whose acquisition has transformed eukaryotic life more than a billion years ago. Established bacterial endosymbionts are found all across the eukaryotic kingdom and provide their hosts with new physiological capabilities. Co-evolution often results in highly complementary functions encoded by host and endosymbiont genomes indicating integration of metabolic processes between the partner organisms. However, the molecular mechanisms underlying host-endosymbiont interactions are largely unknown. While endosymbionts where initially expected to exchange only metabolites with their hosts, recently, also host-encoded proteins were shown to traffic to the endosymbiont in various systems. Some of these symbiont-targeted proteins regulate the symbiont growth and physiology, but cellular targets, mode of action, and targeting mechanisms are currently unknown due to a lack of efficient molecular tools that enable functional studies of endosymbiotic systems. Here we show that the trypanosomatid *Angomonas deanei* containing a single, vertically inherited β-proteobacterial endosymbiont in its cytoplasm is readily amenable to genetic manipulation. The rapid growth, availability of full genome and transcriptome sequences, ease of transfection and high frequency of homologous recombination have allowed us to stably integrate transgenes into the *A. deanei* nuclear genome, efficiently generate null mutants, and perform protein localization studies by heterologous expression of EGFP fused to various targeting signals. Combining proteomic analysis with the newly developed molecular tools were key for demonstrating the routing of the host ETP1 protein to the endosymbiont suggesting the existence of an endosymbiont-sorting machinery in *A. deanei*. 
One billion years of evolution since the separation of kinetoplastids, including *Trypanosoma brucei*, from other eukaryotes has resulted in novel molecular and cellular features. In particular, the transcription of protein coding genes is polycistronic and an understanding of the initiation of transcription of these genes clusters by RNA polymerase II (pol II) has been elusive. Previous work has suggested that transcription might start in Strand Switch Regions (SSRs) between transcription units, however SSRs are large and it is not clear whether or how transcription starts in SSRs.

To investigate the occupancy of RNA pol II in the *T. brucei* genome we performed a series of chromatin immunoprecipitation combined with massive parallel sequencing (ChIP-Seq) assays. The high resolution of this technique allowed us to accurately define regions where RNA pol II accumulates. Our results revealed a significant accumulation of RNA pol II in 306 peaks along the genome, most of them associated with SSRs (52.6%), compared to intergenic regions (27.4%) and coding sequences (19.9%). Sequences occupied by RNA pol II from chromosome 7 were analyzed for promoter activity using a reporter gene assay after transient transfection. Functional promoters were identified within the SSRs as independent dual promoters driving each one the polycistronic transcription units. One of the sequences was further analyzed first by sequential deletions to define the smallest region necessary for fully transcription efficiency and then by mutations across this fragment. We determined the transcription start site (+1) by primer extension and found that main regulatory elements were located downstream of the +1. Thus, the potential of these regions as promoter sequences is being finally recognized and sequence-defined promoters within the SSRs are demonstrated.
Queuosine: The role of an essential tRNA modification in parasitic protist

*Trypanosoma brucei*

Kulkarni, S.¹, Stanzl, H.¹, Kessler, A.², Běhálková, V.¹, Alfonzo, J.D.², Paris, Z.¹

¹Institute of Parasitology, Biology Centre ASC, České Budějovice, Czech Republic
²The Ohio State University, Columbus, Ohio, USA

Transfer RNAs (tRNAs) are a type of non-coding RNAs, which are extensively modified post-transcriptionally, in order to increase their structural stability or fidelity. In particular, the modifications present in the anti-codon loop, have a crucial role in accurate codon selection and translational frameshifting prevention. Queuosine (Q), a hyper modified guanosine, is one such modification, that is found at the wobble position 34 of tRNAs that contain a 5’-GUN-3’ anticodon sequence (His, Asp, Asn, Tyr). Although Q is present in nearly all forms of life, its exact physiological role remains unclear. We aim to study the enzyme responsible for incorporation of Q into the tRNAs, known as tRNA guanine transglycosylase (TGT), in *Trypanosoma brucei*, a protozoan parasite that causes sleeping sickness in humans and nagana in livestock. We have identified two TGT homologs in *T. brucei*, TbTGT1 and TbTGT2, using bioinformatics approaches. Since preliminary data shows that TGT is uniquely essential for the growth of the parasite, while literature so far suggests that its absence has no noticeable phenotype in the mammalian hosts of *T. brucei*, TGT becomes an ideal target for drug development against the disease. We utilize techniques, such as RNA interference (RNAi), *in situ* tagging of proteins, and other methods molecular biology, to comprehensively study the TGT enzyme system, including its structure, kinetics, localization and its relation to the pathogenicity of the parasite in its mammalian host.
Increased prevalence of human cutaneous leishmaniasis in Israel and the Palestine is caused by the recent emergence of genetically similar strains of *Leishmania tropica*

*Azmi, K.¹ ², Krayter, L.³, Schnur, L.F.⁴, Schöbian, G.³*

¹Al-Quds Nutrition and Health Research Institute, Faculty of Medicine, Al-Quds University, Abu-Deis, West Bank, Palestine
²Department of Biochemistry and Molecular Biology, Faculty of Medicine, Al-Quds University, East Jerusalem, Abu-Deis, Palestine
³Institute of Microbiology and Hygiene, Charité University Medicine Berlin, Berlin, Germany
⁴Kuvin Centre for the Study of Infectious and Tropical Diseases, IMRIC, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Twelve unlinked microsatellite markers were used to determine the microsatellite profiles of 50 newly and 46 previously typed strains of *L. tropica* from various Israeli and Palestinian foci. Their microsatellite profiles were compared to those of 99 previously typed strains of *L. tropica* from 15 countries and of eight strains of *L. aethiopica*. Israeli and Palestinian strains of *L. tropica* fell into three different clusters, one of which contained 75 of the 96 Israeli and Palestinian strains. This population separated from all the others at the first hierarchical level by Bayesian statistics and formed a distinct monophyletic group on applying genetic distance and allele frequency analyses. The second cluster contained ten Israeli strains from a specific focus north of the Sea of Galilee, which were previously shown to differ from all other strains of *L. tropica* in their serological, biochemical and molecular biological parameters. This cluster was closely related to clusters comprising strains of *L. tropica* from Africa. Four Israeli and five Palestinian strains fell into different genetic entities mostly related to strains from Asian foci of CL. The expansion of one population in Palestinian and Israeli foci of CL comprising almost all strains of *L. tropica* isolated since 1996 which differ clearly from all other strains of whatsoever origin is also supported by a next-generation multilocus sequence analysis based on whole-genome sequence information.
Worldwide population genetic structure of the *Leishmania donovani* complex based on multi-locus microsatellite and whole-genome SNP variation


¹Institute of Microbiology and Hygiene, Charité University Medicine Berlin, Berlin, Germany
²Unit of Molecular Parasitology, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
³School of Mathematics, Applied Mathematics, and Statistics, National University of Ireland, Galway, Ireland
⁴Département Systématique et Evolution, Muséum National d'Histoire Naturelle, Paris, France
⁵Wellcome Trust Sanger Institute, Genome Campus, Hinxton, CB10 1SA, United Kingdom

Parasites of the *L. donovani* species complex (LDC) cause one the most deadly vector-borne parasitic human diseases, visceral leishmaniasis (VL). Considered anthropoontic, *L. donovani* causes VL epidemics on the Indian subcontinent and in East Africa. *L. infantum*, which is distributed in the Mediterranean Basin, Middle, Central, and East Asia and has managed to spread to South America, is flexible in host-specificity and has a zoonotic life cycle involving canines as main reservoir host. Recent classification confirmed the LDC as taxonomic unit complex comprising isolates of *L. donovani* and *L. infantum*. In some endemic areas both taxa have been reported as causative agents for VL. Before the era of high throughput whole-genome sequencing, multi-locus microsatellite typing (MLMT) approaches had proven most discriminatory for investigating the population structure of the LDC, but these studies were limited by the number of isolates involved or foci included. This is the first study investigating the worldwide population structure of the LDC compiling a representative set of 845 isolates from different clinical forms of the diseases and hosts, from the Old and the New Worlds in order to understand their relationships and the significance of population structure on a global perspective. Furthermore, a subset of 125 fully sequenced isolates, reflecting the worldwide distribution of the LDC, was investigated in a next-generation multi-locus sequence approach (ng-MLSA) including SNPs identified in 235 coding regions for phylogenetic analysis. Both microsatellite and SNP data sets were analysed using, in general, the same population genetic tools, based on different analytic algorithms, e.g. Bayesian clustering statistics and genetic distance analyses. The MLMT study revealed three monophyletic genetic groups of *L. donovani*, a relatively homogeneous population on the Indian subcontinent (ISC) and two populations in East Africa distinctive from each other and from the ISC population. *L. infantum* parasites presented population structures at different hierarchical levels. The ng-MLSA approach has, in general, corroborated the population structures obtained with MLMT for the larger data set. Depending on the genetic markers used, genotypes were identified that precluded clear separation of *L. donovani* and *L. infantum* giving new insights on the diversification of the LDC.
The role of biting midges in avian trypanosomes transmission

Svobodová, M.1, Dolník, O.1, Čepička, I.2, Rádrová, J.1

1Department of Parasitology, Faculty of Science, Charles University in Prague
2Department of Zoology, Faculty of Science, Charles University in Prague

Avian trypanosomes have been shown to form three major clades: T. cf avi um, T. corvi/T. culicavium, and T. bennetti. Confirmed vectors are black flies (Simuliidae); hippoboscids (Hippoboscidae) and mosquitoes (Culicidae); vectors of T. bennetti remain unknown. Our aim was to clarify the role of biting midges as vectors of avian trypanosomes. Based on our survey of ornithophilic bloodsucking insects, we supposed that midges do not act as vectors of avian trypanosomes since no infected midge has been found. Laboratory-bred Culicoides nubeculosus have been artificially fed on different trypanosome strains, showing high susceptibility for T. bennetti (90-100% infected, heavy infections in 50-87% of individuals), and for T. avium as well (85 % infected, heavy infections in 55 %). On the other hand, C. nubeculosus was not susceptible to T. culicavium.

Infectivity of trypanosome stages from midges has been tested using canaries (Serinus canaria). T. bennetti strains were infective only after subcutaneous inoculation while T. avium transconjunctivally or perorally. Parasites were localized in the abdominal midgut and hindgut, resp., of the insect vector.

We confirm the permissiveness of C. nubeculosus biting midges for avian trypanosomes from both T. bennetti and T. avium clades; natural vector species remain to be confirmed.
The unexpected diversity of monoxenous trypanosomatids

Votypka, J.1,2, Kostygov, A., Maslov, D., Yurchenko, V., Lukes, J.

1Department of Parasitology, Faculty of Sciences, Charles University, Prague, Czech Republic
2Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Ceske Budejovice, Czech Republic

While dixenous trypanosomatids of the genera *Trypanosoma* and *Leishmania* represent one of the most dangerous parasites for humans and domestic animals, their "unimportant" monoxenous relatives, which have been overlooked for a long time, recently become model organisms for studies of diversity and host–parasite associations. Biodiversity of trypanosomatids is one of the highest among parasitic protists; however, the classification of the family Trypanosomatidae is not finalized yet and in the past was quite often confusing. The former system was established about half a century ago based on morphology and differences in life cycle. However polymorphism makes morphological features basically unusable, molecular data did not correspond with traditional taxonomical system in some cases, and the host specificity remains to be one of the biggest and the most challenging mysteries of trypanosomatid biology. Despite recent advances, the systematic of Trypanosomatidae was far from being consistent with the known phylogenetic affinities within this group. In current days, the taxonomy is being redefined by using molecular data to keep pace with the discovery of diversity of kinetoplastid flagellates especially in the situation, when several new genera and subfamilies were established.

Over the past years, clearly para-/polyphyletic status of several genera of the family Trypanosomatidae has been clarified considerably. Currently, all three dixenous genera, *Trypanosoma, Leishmania, and Phytomonas*, are recognized as monophyletic, and other recently published studies, following the rule of the modern systematics, have originated monophyly of monoxenous genera. The genus *Herpetomonas* has been redefined and the genus *Lafontella* has been chipped off; *Angomonas* and *Strigomonas* have been re-erected and together with the newly described genus *Kentomonas* form the endosymbiont-harboring subfamily Strigomonadinae; after a quite knotty peripety even the genus *Wallacemonas* has found the final position and together with the newly described genera, *Sergeia, Blechomonas*, and *Jaenimonas*, enriched the current phylogenetic tree of trypanosomatids. Our next effort will be focused on the remaining distinct genus *Blastocrithidia* and its relatives, of which re-definition would be in place. To further clarify the internal structure and composition of the tree, the slowly-evolving species have been incorporated into the subfamily Leismaniinae, which accommodates five genera (*Leishmania, Leptomonas, Crithidia, Novymonas*, and *Lotmaria*) and currently represents the latest problematic group within the whole family Trypanosomatidae.
eTryps

Kostygov, A.\textsuperscript{a,b}, Votýpka, J.\textsuperscript{c,d}, Maslov, D.\textsuperscript{e}, Lukeš, J.\textsuperscript{d,f}, Yurchenko, V.\textsuperscript{a,d}

\textsuperscript{a}Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czech Republic  
\textsuperscript{b}Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia  
\textsuperscript{c}Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic  
\textsuperscript{d}Biology Centre, Institute of Parasitology, Czech Academy of Sciences, České Budějovice (Budweis), Czech Republic  
\textsuperscript{e}Department of Biology, University of California at Riverside, Riverside, CA, USA  
\textsuperscript{f}Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic

Because of the numerous taxonomic revisions in the last few years (new species, new genera, new subfamilies), the up-to-date catalog of Trypanosomatidae is a necessity. We would like to invite the parasitological community to contribute to such an endeavor. The on-line catalog should be manually curated, updated frequently, and include a built-in feature allowing species identification based on 18S ribosomal and/or SL RNA gene sequences.
A novel alternative genetic code in *Blastocrithidia* sp.

Záhonová, K.1,2*, Kostygov, A.1*, Ševčíková, T.1, Yurchenko, V.1,2, Eliáš, M.1,2

1Life Science Research Centre, Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10, 710 00 Ostrava, Czech Republic
2Institute of Environmental Technologies, Faculty of Science, University of Ostrava, Chittussiho 10, 710 00 Ostrava, Czech Republic.
*these authors contributed equally to this study

A limited number of alternative genetic codes have been described for eukaryotic nuclear genomes, most involving reassignment of one or two termination codons as sense codons. Here we describe a novel genetic code variant that we discovered in a clade of Kinetoplastea classified as the genus *Blastocrithidia*. In these parasites all three standard termination codons have been reassigned. UGA now encodes tryptophan, and UAG and UAA (UAR) specify glutamate. Furthermore, UAA and less frequently UAG at the same time serve as *bona fide* termination codons. Surprisingly, the changed genetic code has not acquired modifications of the release factor eRF1 that mediate termination codon reassignments in other eukaryotes, indicating a unique molecular mechanism behind the code change in *Blastocrithidia*. Our results expand the space of a biologically possible variation in an essential molecular mechanism.
Mitochondrial gene expression is responsive to starvation stress and developmental transition in *Trypanosoma cruzi*

*Shaw, A.K., Kalem, M., Zimmer, S.L.*

Department of Biomedical Sciences, University of Minnesota Medical School, Duluth campus, Duluth, MN, USA

Dixenous trypanosomatids contend with differing metabolic and oxidative stresses in their various host environments. Their mitochondrial genomes encode electron transport chain (ETC) components and other genes essential for mitochondrial maintenance. Despite the clear case for widely exploring the malleability of this genome’s expression, most studies are restricted to life stage changes in *Trypanosoma brucei*. *T. brucei* represents an extreme example of mitochondrial gene expression remodeling: where some products present in the procyclic stage are entirely absent in the bloodform stage. Our overarching focus is the responsiveness of the mitochondrial genome of dixenous trypanosomatids in general to developmental and environmental stimuli. Unlike *T. brucei*, some trypanosomes likely maintain a level of ETC function throughout their life cycles. However, their mitochondrial genomes may require different developmental modulation, or else may be responsive to environmental stimuli. We focus on the causative agent of Chagas disease, *Trypanosoma cruzi*. We determined mitochondrial gene expression responses to nutrient and developmental perturbations in insect stage axenic cultures of the CL Brener reference strain at the RNA level. We found that multiple mature mitochondrial mRNAs increase in abundance in response to nutrient stress in epimastigotes. However, CYb mRNA appears developmentally regulated. General starvation, not the removal of a specific nutrient, is the primary stimulus for mRNA increases that are rapid and reversible. As mRNA and protein levels of nuclear-encoded ETC subunits that associate with mitochondrial gene products are not obviously co-regulated, the biological consequence of mitochondrial mRNA abundance increases remains elusive. Finally, *T. cruzi* exhibits a high degree of intraspecies genetic divergence, particularly of the mitochondrial genome. We are now investigating the universality of our results with other *T. cruzi* strains. In summary, we show that *T. cruzi* mitochondrial gene expression responds to developmental and/or environmental stresses in the insect stages. An intriguing possibility is that this also occurs in its mammalian stages.
Posters
First report of isolation and molecular characterization of *Crithidia fasciculata* from a cutaneous lesion from an immunocompetent patient in Peru

*Boucinha, C.*, *Morelli, K.A.*, *dos Santos-Pereira, S.M.*, *Martinez, E.*, *Durán, P.*, *Brandão, A.A.*, *d'Avila-Levy, C.M.*

Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil.
Universidade do Estado do Rio de Janeiro, UERJ, Rio de Janeiro, Brazil
Instituto de Investigación en Salud y Desarrollo, La Paz, Bolivia

Trypanosomatids are flagellated protozoa that present two distinct groups: (1) Monoxenic that are characterized by having its life cycle in a single invertebrate host and are nonpathogenic to mammals; and (2) Heteroxenic that alternates its life cycle between an insect vector and a vertebrate host or plant. The last group encompasses trypanosomatids that impact on public health and promote economic losses in agriculture and livestock. Although monoxenic trypanosomatids are assumed as non-pathogenic to vertebrates, they recently started to attract attention due to the extensive biological diversity, ubiquitous distribution, potential impact on their insect hosts, to support evolutionary studies, and are important models for understanding the biological behavior, biochemistry and genetics of the pathogenic counterparts. They can also be used as vaccine candidates and as platforms for the production of eukaryotic proteins. Notwithstanding, there are some reports in the literature of infections in healthy and immunosuppressed patients caused by monoxenic trypanosomatids. Here, we analyzed an isolate obtained from a skin lesion compatible with cutaneous leishmaniasis from a Peruvian farmworker, which had no immunosuppressive disease and was attended at a hospital in Cusco (Peru). However, studies performed at Instituto de Investigación en Salud y Desarrollo, La Paz, Bolivia, revealed that the isolated parasite firstly identified in Peru as *Leishmania braziliensis*, presented a biological behavior incompatible with this species. This isolate was sent to Protozoa Culture Collection at Fiocruz (www.colprot.fiocruz.br) for molecular identification. The molecular targets gGAPDH, V7V8 SSU rRNA and COI revealed that this isolate is actually an isolate of *Crithidia fasciculata*. For all markers, the genetic distance was between 0 and 0.01% for *C. fasciculata*, *Crithidia luciliae*, *Crithidia ricardoi*, *Crithidia guilhermei*, the clinical isolate (all available in culture at COLPROT) and a *C. fasciculata* sequence available in Genbank. Therefore, we suggest the synonymization of all these species, following the guidelines proposed by Votypka et al., 2015. In addition to these findings, preliminary results show that this strain is able to infect murine peritoneal macrophages *in vitro*. Currently, we are assaying the infection capability of this isolate in vertebrate and invertebrate host models, Balb/c mice and *Lutzomyia longipalpis*, respectively.
A comparative analysis of metabolic and sex-related genes in *Bodo saltans* and parasitic trypanosomatids

Butenko, A.¹, Opperdoes, F.R. ², Filatov, D.³, Eliáš, M.¹, Yurchenko, V.¹, ⁴, Flegontov, P.¹, ⁴, Lukeš, J. ⁴, ⁵, ⁶

¹University of Ostrava, Faculty of Science, Life Science Research Centre, Ostrava, Czech Republic
²de Duve Institute, Université catholique de Louvain, Brussels, Belgium
³University of Oxford, Department of Plant Sciences, Oxford, UK
⁴Biology Centre ASCR, Institute of Parasitology, České Budějovice, Czech Republic
⁵University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic
⁶Canadian Institute for Advanced Research, Toronto, Ontario, Canada

Kinetoplastids (Euglenozoa, Kinetoplastea) is a widespread and important group of single-celled eukaryotes, which includes free-living 'bodonids' and parasitic trypanosomatids. Trypanosomatids can be restricted to one host (monoxenous) or have a life cycle involving two hosts (dixenous). The latter group contains *Trypanosoma* and *Leishmania* species pathogenic for vertebrates and plant pathogens *Phytomonas*. Comparative analysis of the genome of a free-living eubodonid, *B. saltans* along with several trypanosomatid genomes may provide an insight into metabolic gains and losses that enabled the evolutionary transition to parasitism. Our analysis of thirteen kinetoplastid genomes (including the genomes of *Leptomonas pyrrhocoris*, *Leptomonas seymouri*, *Blechomonas ayalai* and *Paratrypanosoma confusum* sequenced by our group) revealed that the adoption of the parasitic lifestyle led to the loss of more than 50% of protein coding genes, resulting in the loss of complete metabolic pathways, such as lysine and histidine catabolism and aromatic amino-acid degradation. The acquisition of novel genes involved in pteridine reduction, threonine dehydration, the urea cycle, protection against ROS, and diaminopimelate metabolism is observed along with gene losses. Interestingly, despite the presence of all glycolytic pathway genes in *B. saltans*, the transcriptome sequence data revealed no evidence for their expression. We also searched for meiosis-associated genes and performed the analysis of recombination using the genomes of 6 *L. pyrrhocoris* isolates originating from Central America. The results indicate the presence of meiosis-related genes in *L. pyrrhocoris* along with the absence of recombination (very low levels of recombination were detected for several scaffolds).
Streamlined mitochondrial genome of the euglenozoan *Euglena gracilis*

*Dobáková, E.*,1,2 *Flegontov, P.*,1,3 *Skalický, P.*,1,4 *Lukeš, J.*,1,4,5

1Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, (Budweis), Czech Republic
2Departments of Biochemistry and Genetics, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
3Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czech Republic
4Faculty of Science, University of South Bohemia, České Budějovice (Budweis), Czech Republic
5Canadian Institute for Advanced Research, Toronto, Canada

In this study, we describe the mitochondrial genome of the excavate flagellate *Euglena gracilis*. Its gene complement is reduced as compared to the well-studied sister groups Diplonemae and Kinetoplastea. We have identified seven protein-coding genes: three subunits of respiratory complex I (*nad1, nad4* and *nad5*), one subunit of complex III (*cob*), and three subunits of complex IV (*cox1, cox2* and a highly divergent *cox3*). Moreover, fragments of ribosomal RNA genes have also been identified. Genes encoding subunits of complex V, ribosomal proteins and tRNAs were missing, and are likely located in the nuclear genome. While mitochondrial genomes of diplonemids and kinetoplastids possess the most complex RNA processing machineries known, including trans-splicing and editing of the uridine insertion/deletion type, respectively, our transcriptomic data suggest their total absence in *E. gracilis*. This finding supports a scenario in which the complex mitochondrial processing machineries of both sister groups evolved relatively late in evolution from a streamlined genome and transcriptome of their common predecessor.
In vitro biological activity and in vivo therapeutic profile of ursolic acid on amastigotes forms of Leishmania braziliensis and against experimental cutaneous leishmaniasis

Furini, J., Gonçalez, C., Albuquerque, S.

School of Pharmaceutical Science of Ribeirão Preto – USP, Brazil

Introduction. Leishmaniasis is a group of tropical diseases caused by species of protozoan parasites belonging to the genus Leishmania. It affects around 12 million people in 88 countries. Historically, the chemotherapy of leishmaniasis has been based on the use of toxic heavy metals, particularly antimony compounds, pentamidine and amphotericin B. Previously, our group evaluated the triterpenes acids activity against trypomastigotes forms of the Trypanosoma cruzi and found good results.

Objective. The aims of this work were evaluate the in vitro leishmanicidal effect of ursolic acid on amastigotes forms of L. braziliensis and investigate its therapeutic potential against experimental cutaneous leishmaniasis.

Methods. In vitro assay was performed in 96-well microplates where culture of cell lines P338D1 grown in RPMI-1640 medium. Promastigotes forms of L. braziliensis 3222, was added in the ratio of 1:10 (cell:parasite) and incubated for 24 h at 37° C with 5% CO2. Ursolic acid was added to obtain final concentrations of 0.5, 2, 8, 32 and 128 μM, in triplicates. The parasites were quantified by cytometry based on image methodology (TALI – Life Technologies). To perform in vivo experiments, were used 20 male mice (5 animals/group) inoculated subcutaneously at the tail base with 1x10⁶ promastigotes forms of L. braziliensis. The proposed oral treatments (20 days) started after the pre-patent period. The drugs used in the trials were the ursolic acid (UA) and its derivative solid dispersion (UASD). During the treatment period, the extensions of the lesions were evaluated using a digital caliper every 3 days.

Results. UA showed high leishmanicidal activity mainly at concentration of 128 μM (IC₅₀ = 5,126 μM). The percentages of lysis in this concentration was 90.21%. Confirming the in vitro results, UA and UASD showed a reduction the mean diameter of lesions, with lower variations during treatment when compared to the negative control.

Conclusions. These results indicate high leishmanicidal activity of UA, and indicate that pharmacotechnical modifications potentiated the desired effect against cutaneous leishmaniasis.
Diversity and co-infections of trypanosomes and trypanoplasms in freshwater fishes

Grybchuk-Ieremenko, A.\textsuperscript{1}, Kostygov, A.\textsuperscript{Y.1,3}, Losev, A.\textsuperscript{1,2}, Lukeš, J.\textsuperscript{4,5}, Yurchenko, V.\textsuperscript{1,4}

\textsuperscript{1}University of Ostrava, Faculty of Science, Department of Biology and Ecology, Ostrava
\textsuperscript{2}Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv, Ukraine
\textsuperscript{3}Zoological Institute, Russian Academy of Sciences, Saint Petersburg, Russia
\textsuperscript{4}Biology Centre, Institute of Parasitology, Czech Academy of Sciences, České Budějovice,
\textsuperscript{5}Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

This study represents the eight year research of freshwater fish parasites - Trypanosoma sp. and Trypanoplasma sp. Totally 1373 fish specimens (8 species) from the vicinity of Kyiv (Ukraine) were examined for the presence of trypanosomes and trypanoplasms. Using 18S rRNA gene sequences it was found that four fish species (pike, bream, spined loach and European perch) were co-infected by two different trypanosome species. Cases of co-infection by trypanoplasms have been documented as well. Significant variation was observed in the level of infection, which ranged from 24% in freshwater bream to 100 % in spined loach. In the majority of cases the infections with trypanosomes and trypanoplasms were asymptomatic, while the level of parasitaemia ranged a lot from mild infections in pikeperch to heavy ones in spined loach. Results of our work show the necessity of using clonal lines derived from laboratory cultures for further molecular taxonomic studies of fish trypanosomes and trypanoplasms in order to avoid possible ambiguities in future.
Morphology and the host-specificity of *Herpetomonas tarakana*, recently described monoxenous trypanosomatid from cockroaches

_Havlová, J.\textsuperscript{a}, Kostygov, A.\textsuperscript{b,c}, Yurchenko, V.\textsuperscript{b,d}, Votýpka, J.\textsuperscript{a,d}_

\textsuperscript{a}Department of Parasitology, Faculty of Science, Charles University, 128 44 Prague, Czech Republic,
\textsuperscript{b}Life Science Research Centre, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czech Republic
\textsuperscript{c}Zoological Institute, Russian Academy of Sciences, 199034 St. Petersburg, Russia
\textsuperscript{d}Biology Centre, Institute of Parasitology, Czech Academy of Sciences, 370 05 České Budějovice (Budweis), Czech Republic

Recently, we have described a new species of monoxenous trypanosomatid belonging to the genus *Herpetomonas, Herpetomonas tarakana*, which was isolated from the intestinal tract of a sylvatic cockroach (*Ectobius lapponicus*). The new species is described based on sequence analyses (small subunit (SSU) rRNA and glycosomal glyceraldehyde 3-phosphate dehydrogenase (gGAPDH) genes), microscopic analyses (light and electron microscopy) and supposed host specificity (see Yurchenko et al. 2016). In the predominant cell morphotypes of *H. tarakana*, promastigotes and choanomastigotes, we analyzed unique morphological structures - a deep flagellar pocket, (hemi)desmosomes on the contact area between a flagellum and a membrane and microtubules surrounding the flagellar pocket. By using axenic cultures derived from sylvatic cockroaches we studied the host specificity of *H. tarakana*. Experimental infections of synanthropic and sylvatic cockroaches were performed and obtained results demonstrate that *H. tarakana* is highly host-specific monoxenous species.
A comparison of the peritrophic matrix in different sand flies species and its role in the Leishmania development

Homola M., Sadlova J., Volf P.

Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

**Introduction:** The sand fly peritrophic matrix (PM) is an acellular envelope, composed of chitin fibers and proteins, which surrounds the ingested blood meal. It protects the midgut epithelium against abrasion, toxic substances and pathogens and improves the digestion of food by compartmentalization of the gut into endo- and ectoperitrophic spaces. This study is focused to the role of the PM in vector competence of sand flies for Leishmania parasites.

**Methods:** We compared the morphology and kinetics of the PM in two natural vectors of *L. donovani* (*Phlebotomus argentipes, P. orientalis*) and two refractory species (*P. papatasi, Sergentomyia schwetzi*). Blood fed females were dissected at ten time intervals post bloodmeal and their midguts were examined for the presence of the PM using the light microscopy. For the histological study, the females with fully developed PM were embedded in JB-4 and histological sections (4-6 μm) were stained with haematoxylin and eosin.

Experimental infections of *S. schwetzi* with GFP transfected *L. major* were done using membrane-feeding method and the intensity and localization of Leishmania infections in the midgut were determined by dissection and examination by light and fluorescent microscopy.

**Results/Conclusions:** The four sand fly species did not differ substantially in the morphology of the PM, in all species the anterior plug secreted by the thoracic part of the midgut was formed and the PM was evenly closed on the posterior end. On the other hand, the kinetics of formation and degradation of the PM was species-specific. Importantly, the period between the breakdown of the PM and defecation of bloodmeal remnants strikingly differed among sand fly species: it lasted on average 21h, 48h and 38h in *P. argentipes, P. orientalis* and *P. papatasi*, respectively, while in *S. schwetzi* this interval was significantly shorter (3h). We suppose this is the cause of refractoriness of *S. schwetzi* to Leishmania parasites - promastigotes developing in *S. schwetzi* do not have enough time to attach to the midgut and are defecated with blood meal remnants. This hypothesis was confirmed by addition of the exogenous chitinase to the infective dose which resulted in artificial degradation of the PM. Under these conditions the Leishmania parasites were able to survive the defecation and even colonize the stomodeal valve of *S. schwetzi.*
Flagellar transporter TbHrg conveys heme and controls differentiation in procyclic *Trypanosoma brucei*


1Institute of Parasitology, Biology Center, Czech Academy of Sciences, 37005 České Budějovice (Budweis), Czech Republic
2Institute of Microbiology, Czech Academy of Sciences, 37981 Třeboň, Czech Republic
3Faculty of Sciences, University of South Bohemia, 37005 České Budějovice (Budweis), Czech Republic
4Department of Animal Health, Unit of Veterinary Protozoology, Institute of Tropical Medicine Antwerp (ITM), Antwerp, Belgium
5Institut de Biologie et de Médecine Moléculaires, Université Libre de Bruxelles, B6041 Gosselies, Belgium

We have performed functional characterization of heme transporter *TbHrg* in the procyclic stage of *Trypanosoma brucei*. Depletion of *TbHrg* in knock-out cells resulted in slower proliferation, a decrease of cellular heme and marked changes of cellular morphology, consistent with a switch from the procyclic stage to proventricular mesocyclic trypomastigotes. Moreover, the spectrum of procyclins was extensively remodeled by the expression of *TbHrg*, which is more essential during heme deprivation. The early procyclin GPEET was decreased in the *TbHrg* knock-out cells and strongly upregulated in the *TbHrg* overexpressing flagellates, whereas the late procyclin EP1 was down-regulated upon *TbHrg* overexpression. The *TbHrg* protein localizes to the flagellar membrane, with more intense labeling towards the flagellar pocket. We postulate that trypanosomes sense the level of heme *via* the flagellar *TbHrg* and that heme represents a key environmental stimulus triggering inter-stagial transformation of this important human parasite, probably through metabolic remodeling.
Species identification and phylogenetic analysis of *Leishmania* isolated from Xinjiang Autonomous Region, The People’s Republic of China


¹Center for Parasitic Organisms, State Key Laboratory of Biocontrol, Key laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Guangzhou 51027, P.R. China and Key Laboratory of Tropical Disease Control and Prevention of the Ministry of Education, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou 510080, P.R. China

²The Center for Laboratory Animal Research of Xinjiang, Urumqi 830002, P.R. China

³Laboratory of Molecular Protozoology, Life Science Research Center, University of Ostrava, Ostrava, Czech Republic

#equal contribution

Visceral leishmaniasis (VL) is one of the six major tropical diseases declared by WHO. This disease has been successfully controlled although it remains endemic in northwestern China. Xinjiang Autonomous Region is currently the most endemic region with prevalence of both anthropoanotic VL (AVL) and desert type zoonotic VL (DT-ZVL). Here, we report the identification of the *Leishmania* species isolated from patients, sand-fly vectors and the suspected reservoir animal Tarim hare (*Lepus yarkandensis*) in DT-ZVL and from patients in AVL. ITS1, HSP70 and NAGT sequences were obtained for species determination and phylogenetic analysis. Sequence analysis demonstrated that the all *Leishmania* isolates tested in this work belong to the *L. donovani* complex. Two epidemiological types of this disease are caused by two distinct groups of parasite; isolates from AVL are close to *L. infantum* while those from DT-ZVL are close to *L. donovani*. Isolates from DT-ZVL are unique and different from those *L. donovani* isolates reported worldwide, suggesting that these parasites have been restricted here rather than currently introduced. Our data further support that Tarim hare is a reservoir for the DT-ZVL, which requires a new strategy to control human infection from this animal.
The kinetoplast minicircle transcriptome of *Leptomonas pyrrhocoris*

Gasparyan, A.A.¹, Gerasimov, E.S.¹, Litus, I.A.¹, Kolesnikov, A.A.¹, Flegontov, P.N.²

¹Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
²Faculty of Science, University of Ostrava, Ostrava, Czech Republic

We used Next Generation Sequencing (NGS) methods to analyze kinetoplast minicircles in *Leptomonas pyrrhocoris*. Minicircle transcripts were assembled *de novo* from Illumina reads, and then transcript integrity and minicircle copy number was checked by mapping genomic Illumina reads. The average size of minicircles in this species is about 1150 – 1300 bp. Comparison of the sequences of the minicircle conservative region revealed the high level of sequence heterogeneity.

There are about 20 groups of major (abundant) minicircle variants and a lot of rare variants. All of them have typical conserved sequences CSB I – III. It was a surprise to obtain high coverage with transcriptome reads in this region of minicircles. In general, minicircles were transcribed in full. We assembled a few full-length minicircles using transcriptomic reads, and confirmed the assemblies with PCR using specific primers and by sequencing cloned minicircles. The second surprise was the fact that a majority of *L. pyrrhocoris* minicircles has a dimeric structure. There are two symmetric conservative regions per circle, like in minicircles of *Crithidia fasciculata* and *Herpetomonas samuellpessoai*.

To our knowledge, this is the first study of minicircles in a monoxenous insect trypanosomatid that employs NGS technology.
A phytomonad, which is no longer a plant parasite: secondary monoxenous

*Phytomonas nordicus*

Frolov, A.O.a, Malysheva, M.N.a, Yurchenko, V.b,c, Kostygov, A.Y.a

aZoological Institute of the Russian Academy of Sciences, Universitetskaya nab. 1, St. Petersburg, 199034, Russia
bLife Science Research Centre, Faculty of Science, University of Ostrava, Chittussiho 10, 710 00 Ostrava, Czech Republic
cBiology Centre, Institute of Parasitology, Czech Academy of Sciences, Branišovská 31, 370 05 České Budějovice, Czech Republic

Trypanosomatid *Phytomonas nordicus* parasitizing predatory bug *Troilus luridus* was described at the twilight of the morphotype-based systematics. Despite its monoxenous life cycle, this species was attributed to the dixenous genus *Phytomonas* due to the presence of long twisted promastigotes and development of flagellates in salivary glands. However, these characters were considered to be insufficient to prove phytomonad nature of the species and therefore its description remained virtually unnoticed. We performed molecular phylogenetic analyses using 18S rRNA gene and ITS1/ITS2-containing region and convincingly demonstrated the affinity of *Phytomonas nordicus* to the genus *Phytomonas*. In addition, we scrutinized "the most phytomonad" part of its life cycle, i.e. development in the salivary glands. We argue that in many aspects the life cycle of monoxenous *Ph. nordicus* resembles that of its dixenous relatives exemplified by tomato-parasitizing *Ph. serpens*. 
New insight into catalase gene gain/loss in subfamily Leishmaniinae

Kraeva, N.1, Horáková, E.2, Kostygov, A.Y.1, Kořený, L.2, Lukeš, J.2,3, Yurchenko, V.1,2

1Life Science Research Centre, Faculty of Science, University of Ostrava, 710 00, Ostrava, Czech Republic
2Biology Centre, Institute of Parasitology, Czech Academy of Sciences, 370 05 České Budějovice (Budweis), Czech Republic
3Faculty of Science, University of South Bohemia, 370 05 České Budějovice (Budweis), Czech Republic

Catalase gene is a virtually ubiquitous component of the eukaryotic genomes. It decomposes hydrogen peroxide (H₂O₂) to water and molecular oxygen, thereby protecting cells from the reactive oxygen species (ROS). The recently established subfamily Leishmaniinae is divided into two clades comprising monoxenous (one-host) trypanosomatids species of the genera Crithidia, Lotmaria, Leptomonas and dixenous (two-hosts) Leishmania causing clinically diverse disease leishmaniasis. In our study we showed that in the Trypanosomatidae family catalase gene is present only inside the Leishmaniinae clade. Moreover, within this clade, catalase was restricted to the monoxenous genera and secondarily lost from the dixenous Leishmania spp. This gene was acquired from a bacterium and it was a singular event that occurred in the common ancestor of all Leishmaniinae. Recently, several lines of evidence suggested that generation of diffusible ROS H₂O₂ could play a role in Leishmania differentiation from the extracellular procyclic promastigotes to the infective metacyclics and amastigotes. To shed more light on the gain/loss of a catalase gene in Leishmaniinae and potential involvement of H₂O₂ in parasites' differentiation and survival within macrophages, we overexpressed a monoxenous Leptomonas pyrrhocoris-derived catalase in the dixenous Leishmania mexicana. We have also generated a catalase-null Leptomonas seymouri mutant. As expected, the catalase overexpressing Leishmania cells showed significant increase in their resistant to hydrogen peroxide compared to wild type counterparts (EC₅₀: 0.33 and 1.68 mM H₂O₂, respectively). In order to follow the L. seymouri infection, we also established a catalase-null cell line expressing mCherry fluorescent protein. We believe such multi-dimensional approach will help to understand molecular details of the Leishmania oxidative defense mechanisms and evolution of dixeny in general.
Functional annotation of *Euglena gracilis* mitochondrial proteome


₁Institute of Parasitology, Biology Centre, České Budějovice (Budweis), Czech Republic
₂Faculty of Science, University of South Bohemia, České Budějovice (Budweis), Czech Republic
₃Life Science Research Centre, University of Ostrava, Ostrava, Czech Republic
₄School of Life Sciences, University of Dundee, Dundee, United Kingdom
₅Canadian Institute for Advanced Research, Toronto, Canada

*Euglena gracilis* is a secondary green alga belonging to the phylum Euglenozoa, which also contains kinetoplastid parasites *Trypanosoma* and *Leishmania*. Despite the fact that *E. gracilis* is a well-studied model organism, little is known about its single reticulated mitochondrion. Few previous studies have focused primarily on individual functional groups of mitochondrial proteins, making no attempt to examine mitochondrial proteome as a whole. In this study, we have made an *in silico* prediction of the entire *E. gracilis* mitoproteome based on *de novo* transcriptome sequencing as a part of the *Euglena* genome project. For prediction, we analyzed N-terminal targeting signals (TargetP), similarity to known mitochondrial proteins (BlastP and Blast2GO), and orthologous group information (OrthoMCL) of proteins. The resulting set contains more than 1000 proteins, including subunits of respiratory chain complexes I-V, other components of OxPhos and tricarboxylic acid cycle, mitoribosomal proteins, etc. There are also homologues of *Trypanosoma* editosome components, although RNA editing is not known to occur in *Euglena* mitochondrion; probably these proteins acquired other functions, such as those in mitochondrial RNA processing.
Comparative metabolism of free-living *Bodo saltans* and parasitic trypanosomatids

**Oppendoes, F.R.**, Butenko, A., Flegontov, P., Yurchenko, V., Lukeš, J.

*de Duve Institute, Université Catholique de Louvain, B-1200 Brussels, Belgium*
*Life Science Research Centre, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czech Republic*
*Biology Centre, Institute of Parasitology, Czech Academy of Sciences, 370 05 České Budějovice (Budweis), Czech Republic*
*A.A. Kharkevich Institute for Information Transmission Problems, Russian Academy of Sciences, Moscow, Russian Federation*
*Department of Pathology, Albert Einstein College of Medicine, Bronx, New York 10461, USA*
*Faculty of Science, University of South Bohemia, 370 05 České Budějovice (Budweis), Czech Republic*
*Canadian Institute for Advanced Research, Toronto, Ontario M5G 1Z8, Canada*

Comparison of the genomes of free-living *Bodo saltans* and those of parasitic trypanosomatids reveals that the transition from a free-living to a parasitic life style has resulted in the loss of approximately 50% of protein-coding genes. Despite this dramatic reduction in genome size, *Bodo* and trypanosomatids still share a significant number common metabolic traits: glycosomes; a unique set of the pyrimidine biosynthetic pathway genes; an ATP-PFK which is homologous to bacterial PPy-PFKs rather than to the canonical eukaryotic ATP-PFKs; an alternative oxidase; three phosphoglycerate kinases and two GAPDH isoenzymes; a pyruvate kinase regulated by fructose-2,6-bisphosphate; trypanothione as a substitute for glutathione; synthesis of fatty acids via a unique set of elongase enzymes and an enzyme demonstrated to be involved in the production of acetate by procyclic trypanosomes. Among genes that are present in *B. saltans* and lost in all trypanosomatids are those involved in the degradation of mureine, tryptophan and lysine. Novel acquisitions of trypanosomatids are components of pentose-sugar metabolism, pteridine reductase and bromodomain-factor proteins. In addition, only the subfamily Leishmaniinae has acquired a gene for catalase and the capacity to convert diaminopemelic acid to lysine. Finally, *B. saltans* may have the RNA interference capacity, as do the African trypanosomes, *Leptomonas pyrrhocoris* and *Crithidia fasciculata*, whereas the RNAi pathway is absent in *Blechomonas ayalai*, *Trypanosoma cruzi*, *Phytomonas* spp., and *Leptomonas seymouri*. 
Peters, A.  

1Graham Centre for Agricultural Innovation, (NSW Department of Primary Industries and Charles Sturt University), Wagga Wagga, NSW 2650, Australia  
2School of Animal & Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia  

Prolonged biogeographical isolation has given Australia the world’s most unique vertebrate fauna and freedom from numerous lineages of parasitic protists, including many trypanosomatids. Increasing movement of people and animals and changing vector distributions have caused the historical emergence of trypanosomatid diseases in Australia, with disastrous consequences for some species, and is a threat to Australia’s biosecurity. This poster reviews historical cases of trypanosomatosis causing declines in native mammals and the emergence of leishmaniasis in tropical northern Australia. It highlights trypanosomatids of potential risk to Australia’s biodiversity, agricultural sector and human health.
Conditional expression system is not suitable for developmental studies in *Leishmania*

Ishemgulova A.¹, Kraeva N.¹, Faktorová D.², Podešová L.¹, Lukeš J.²,³,⁴, Yurchenko V.¹,²

¹Life Science Research Centre, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czech Republic
²Czech Academy of Sciences, 370 05 České Budějovice (Budweis), Czech Republic
³Faculty of Sciences, University of South Bohemia, 370 05 České Budějovice (Budweis), Czech Republic
⁴Canadian Institute for Advanced Research, Toronto, ON M5G 1Z8, Canada

The genus *Leishmania* unites parasitic protists of the family Trypanosomatidae causing leishmaniases, several closely related diseases that affect human and animal populations mainly in the tropical and subtropical regions. The clinical manifestations vary from spontaneously healing skin lesions to progressive and potentially fatal visceral infections. Leishmaniases represent a global health problem with over 350 million people at risk and an annual incidence rate of 2–10 million worldwide. Conventional and conditional systems allow for a controlled activation or repression of gene expression in time and space. Such systems are nowadays widely used to analyse a variety of cellular processes in numerous parasites including *Leishmania*.

A T7-driven, tetracycline-inducible system for protein expression was established in a human pathogen *Leishmania mexicana*. The gene expression in this strain is tightly regulated and dose- and time dependent. We believe that it can be widely used by the parasitology community to analyse effects of genes of interest on biology, physiology and virulence of parasites causing cutaneous leishmaniases. This system was used to analyse gene expression profiles during *L. mexicana* differentiation (procyclics, metacyclics, and amastigotes). The transcription/translation of the gene of interest was severely decreased upon *Leishmania* differentiation into metacyclic and amastigotes. However, the same expression profile was documented for the T7 polymerase. The expression was demonstrated to be not locus-specific but dependent on untranslated regions flanking open reading frames of studied genes. We concluded that the previously established conventional gene expression systems might have certain limitations in their common applications.
rSP03B: The salivary recombinant protein used as the universal marker of Phlebotomus perniciosus exposure

Polanska, N.1, Kostalova, T.1, Lestinova, T.1, Willen, L.1, Maia, C.2,3, Sumova, P.1, Vikova, M.1, Fiorentino, E.4, Scalone, A.4, Oliva, G.5, Veronesi, F.6, Cristóvão, J.M.2, Courtenay, O.7, Campino, L.2,8, Gradoni, L.4, Gramiccia, M.4, Volf, P.1

1Dept. Parasitol., Charles University, Prague, Czech Republic
2Med. Parasitol.Unit, GHTM, IHMT, Universida de Nova de Lisboa, Portugal
3Fac. Med. Vet., Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
4Istituto Superiore di Sanità, Rome, Italy
6Dept. Vet. Med., Universityof Perugia, Italy
7WIDER and School of Life Sci., University of Warwick, United Kingdom
8Dept. de Ciências Biomédicas e Medicina, Universidade do Algarve, Faro, Portugal

Leishmania parasites are transmitted by bite of phlebotomine sand flies. During blood feeding, sand fly females inject saliva into the skin of the host and induce host's antibody response. In the repeatedly bitten hosts, antibodies against sand fly saliva persist for weeks and positively correlate with the number of the sand fly bites. Therefore, measuring anti-saliva antibodies is the valuable tool to estimate exposure of hosts to sand flies. As the preparation of whole salivary gland homogenate (SGH) is time-consuming and labor-intensive process, recombinant salivary proteins are proposed as the valid replacement for SGH in epidemiological studies.

Based on our previous studies, we focused to study on 43 KDa yellow-related protein (rSP03B) of Phlebotomus perniciosus. This antigen has been previously proven as a marker of exposure in dogs naturally bitten by P. perniciosus in southern Italy. Here, we tested if this antigen would be useful in geographically distant localities within the entire area of P. perniciosus distribution, where populations of this sand fly are involved in epidemiology of human and canine visceral leishmaniasis. We tested by ELISA 214 sera samples from naturally exposed dogs from south Italy, central Italy and 341 sera samples from Portugal. In all these regions the strong correlation was found between the antibody response against rSP03B and SGH. These results indicate that different populations of P. perniciosus share same antigenic properties of this salivary protein and that 43 KDa yellow-related protein (rSP03B) of P. perniciosus can be used as a general marker of canine exposure to this vector of human and canine visceral leishmaniasis.
The seasonal dynamics of avian trypanosomes in mosquitoes

Rádrová, J., Dolnik, O., Svobodová, M.

Department of Parasitology, Faculty of Science, Charles University in Prague, Czech Republic

To evaluate the role of Culex mosquitoes as potential vectors of avian trypanosomes, a long-term study is conducted in South Moravia, Milovice game preserve. Mosquitoes are captured by CO₂-baited CDC traps in 14-days intervals, identified, and kept in -20 °C until subsequent testing for Trypanosoma presence by Kinetoplastida-specific nested PCR assay. Between March and October 2014 and 2015, resp., 4 397 mosquitoes belonging to 12 species and five genera were caught. The prevailing species were: Culex pipiens, Mansonia richiardii and Culiseta annulata. Mansonia is peaking in the first half of July, while Cs. annulata two weeks later. Cx. pipiens has two peaks, in the first half of July and in the turn of July/August. After heavy rains in September 2014, Aedes vexans appeared in untypically high numbers. Mosquito females (740 pools) were tested by PCR; 96 pools were PCR positive. Total minimal infectious rate was 1.8 % in 2014, and 4.5 % in 2015, resp. From the seasonal point of view, the first T. theileri-infected Culiseta annulata was caught in end of May 2014, and T. theileri-infected Anopheles plumbeus in June 2015. Avian trypanosomes (T. culicaviium) have been detected in Cx. pipiens in July. Resulting sequences represent three groups/species of Trypanosoma: T. theileri s. l., T. culicaviium, and T. avium s. l. Moreover, Paratrypanosoma confusum, Crithidia and Leptomonas spp. were detected. Even though mosquitoes are present on the locality from half of April till beginning of October, Trypanosoma positive mosquitoes occurred only until late August. Transmission thus probably occurs only during a short late spring/summer period.
Patrypanosoma: from free living to a parasitic way of life

Skalický, T.1,2, Dobáková, E.1, Flegontov, P.1,3, Tesařová, M.1, Jirsová, D.1,4, Votýpka, J.1,5,
Yurchenko, V.1,3, Lukeš, J.1,2,6

1Institute of Parasitology, Biology Centre, České Budějovice (Budweis), Czech Republic
2Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic
3Life Science Research Centre, University of Ostrava, Ostrava, Czech Republic
4University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
5Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic
6Canadian Institute for Advanced Research, Toronto, Canada

Paratrypanosoma confusum is a novel uniflagellar monoxenous kinetoplastid discovered in the gut of mosquito Culex pipiens. As the most basal trypanosomatid lineage, it represents a possible evolutionary link between free-living bodonids and obligatory parasitic trypanosomatids. In axenic culture, P. confusum creates three morphologically distinct stages. A promastigote-like motile stage, equipped with a long flagellum, changes under certain conditions into a sessile stage. During this transformation, the paraflagellar rod is dismantled and the external part of the flagellum creates a sticky pad that is used for cell attachment to surfaces. Time-lapse videos proved the flagellum transformation process in sessile stage to be reversible. Furthermore, P. confusum forms an oval, amastigote-like stage with a very short external flagellum when grown on semi-solid agar plates.

P. confusum exhibits social motility behavior with polarized movement of thin rays radiating from center and halting or diverting their movement to avoid contact with other cell projections. Preliminary analyzes suggest that one of signaling molecules moderating this behavior could be biopterin. P. confusum is not forming sessile stage in cultivation medium without biopterin and its absence is also affecting social motility behavior. Moreover, differential expression analyzes performed on transcriptomes of promastigote-like stage and sessile stage revealed over-expression of pteridine transporter genes in the sessile stage.

Presence of RNA interference machinery core gene homologs (Ago1, Piwi1 and DCL1) both in genome and transcriptome together with confirmed presence of dsRNA viruses within P. confusum cells suggests that both dsRNA viruses and RNA interference coexists in ancient species which later evolved to retain only one. Further analyzes of the genome of the monoxenous Paratrypanosoma should provide insight into the emergence of dixenous parasitism of the medically important trypanosomatids.
Diversity and phylogeny of frog trypanosomes

Spodareva, V.V.\textsuperscript{a,b}, Kostygov, A.Y.\textsuperscript{a,b}, Losev, A.\textsuperscript{c}, Grybchuk-Ieremenko, A.\textsuperscript{b}, Votýpka, J.\textsuperscript{d,e}, Yurchenko, V.\textsuperscript{b,d}.

\textsuperscript{a}Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia
\textsuperscript{b}Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czech Republic
\textsuperscript{c}Biology Centre, Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic
\textsuperscript{d}Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

Trypanosomes of amphibia are a neglected group. About 60 species were described on the basis of morphology. Instead of the “one species – one parasite” concept the morphotypical approach was used which led to excessive conservatism. For example, \textit{Trypanosoma rotatorium} was recorded in about 60 species of anurans from Europe, Asia, Africa as well as North and South America. All isolates of frog trypanosomes studied to date form a monophyletic group, which together with trypanosomes of fishes belong to the so-called aquatic clade.

We analyzed 16 isolates from \textit{Pelophylax} \textit{spp.} frogs collected in Ukraine and Czech Republic. Molecular phylogenetic analysis with the use of 18S rRNA gene (~typically 8 per each sample) was complemented with morphological analysis of the cells on the corresponding blood smears. The obtained haplotypes formed groups in different locations within the aquatic clade. Importantly, one of these groups, identified by morphology as \textit{T. loricatum} was found to be sister to the clade of fish trypanosomes, thereby making the frog trypanosomes a paraphyletic group. Two other haplotype groups were found to be related to clades of unidentified Brazilian isolates. Some haplotypes were nested within the clade containing many previously described species of trypanosomes such as \textit{T. ranarum}, \textit{T. rotatorium}, \textit{T. neveulemairei} and others. The correlation of molecular and morphological data demonstrated that different \textit{T. rotatorium}-like isolates not necessarily belong to one species but can be even unrelated. Our results shed light on the evolution of aquatic trypanosomes, demonstrated inconsistency of the traditional morphotype-based approach in their systematics and revealed hidden diversity of these flagellates.
## List of Participants

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alves, Joao Marcelo Pereira</td>
<td>Univerzita São Paulo, Brazil</td>
<td><a href="mailto:jotajj@usp.br">jotajj@usp.br</a></td>
</tr>
<tr>
<td>Boucinha, Carolina</td>
<td>Instituto Oswaldo Cruz, Brazil</td>
<td><a href="mailto:carolbmartins85@gmail.com">carolbmartins85@gmail.com</a></td>
</tr>
<tr>
<td>Butenko, Anzhelika</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:anzhelika.i.butenko@gmail.com">anzhelika.i.butenko@gmail.com</a></td>
</tr>
<tr>
<td>d'Avila-Lévy, Claudia</td>
<td>Instituto Oswaldo Cruz, Brazil</td>
<td><a href="mailto:davila.levy@ioc.fiocruz.br">davila.levy@ioc.fiocruz.br</a></td>
</tr>
<tr>
<td>Dario, Maria Augusta</td>
<td>Instituto Oswaldo Cruz, Brazil</td>
<td><a href="mailto:maria_augustadario@yahoo.com.br">maria_augustadario@yahoo.com.br</a></td>
</tr>
<tr>
<td>De Meeüs, Thierry</td>
<td>Institut de recherche pour le développement, France</td>
<td><a href="mailto:thierry.demeeus@ird.fr">thierry.demeeus@ird.fr</a></td>
</tr>
<tr>
<td>Dobáková, Eva</td>
<td>Institute of Parasitology, Czech Republic</td>
<td><a href="mailto:eva.dobakova@centrum.sk">eva.dobakova@centrum.sk</a></td>
</tr>
<tr>
<td>Dujardin, Jean-Claude</td>
<td>Institute of Tropical Medicine, Belgium</td>
<td><a href="mailto:jcdujardin@itg.be">jcdujardin@itg.be</a></td>
</tr>
<tr>
<td>Eliáš, Marek</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:marek.elias@osu.cz">marek.elias@osu.cz</a></td>
</tr>
<tr>
<td>Ellis, John</td>
<td>University of Technology</td>
<td><a href="mailto:john.ellis@uts.edu.au">john.ellis@uts.edu.au</a></td>
</tr>
<tr>
<td>Field, Mark</td>
<td>University of Dundee, UK</td>
<td><a href="mailto:mfield@mac.com">mfield@mac.com</a></td>
</tr>
<tr>
<td>Flegontov, Pavel</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:pflegontov@gmail.com">pflegontov@gmail.com</a></td>
</tr>
<tr>
<td>Furini, Junior</td>
<td>Univerzita São Paulo, Brazil</td>
<td><a href="mailto:jfurini@gmail.com">jfurini@gmail.com</a></td>
</tr>
<tr>
<td>Ganyukova, Anna</td>
<td>Zoological Institute, Russia</td>
<td><a href="mailto:anna.ganuykova@gmail.com">anna.ganuykova@gmail.com</a></td>
</tr>
<tr>
<td>Gerasimov, Evgeny</td>
<td>Moscow State University, Russia</td>
<td><a href="mailto:jalgard@gmail.com">jalgard@gmail.com</a></td>
</tr>
<tr>
<td>Grellier, Philippe</td>
<td>National Museum Natural History, France</td>
<td><a href="mailto:grellier@mnhn.fr">grellier@mnhn.fr</a></td>
</tr>
<tr>
<td>Grisard, Edmundo</td>
<td>Federal University of Santa Catarina, Brazil</td>
<td><a href="mailto:edmundo.grisard@ufsc.br">edmundo.grisard@ufsc.br</a></td>
</tr>
<tr>
<td>Grybchuk-Ieremenko, Anastasiia</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:Anastasiia1989@gmail.com">Anastasiia1989@gmail.com</a></td>
</tr>
<tr>
<td>Grybchuk, Danyil</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:danilaman@gmail.com">danilaman@gmail.com</a></td>
</tr>
<tr>
<td>Hamilton, Patrick</td>
<td>University of Exeter, UK</td>
<td><a href="mailto:p.b.hamilton@exeter.ac.uk">p.b.hamilton@exeter.ac.uk</a></td>
</tr>
<tr>
<td>Havlová, Jolana</td>
<td>Charles University in Prague, Czech Republic</td>
<td><a href="mailto:jolana.havlova@natur.cuni.cz">jolana.havlova@natur.cuni.cz</a></td>
</tr>
<tr>
<td>Homola, Miroslav</td>
<td>Charles University in Prague, Czech Republic</td>
<td><a href="mailto:mirdahomola@gmail.com">mirdahomola@gmail.com</a></td>
</tr>
<tr>
<td>Horváth, Anton</td>
<td>Comenius University, Slovakia</td>
<td><a href="mailto:horvath@fns.uniba.sk">horvath@fns.uniba.sk</a></td>
</tr>
<tr>
<td>Changmai, Piya</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:jibjibboy@yahoo.com">jibjibboy@yahoo.com</a></td>
</tr>
<tr>
<td>Chen, Yun Fu</td>
<td>Sun Yat-Sen University, P. R. China</td>
<td><a href="mailto:chenyfu@mail2.sysu.edu.cn">chenyfu@mail2.sysu.edu.cn</a></td>
</tr>
<tr>
<td>Ishemgulova, Aygul</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:aishemgulova@gmail.com">aishemgulova@gmail.com</a></td>
</tr>
<tr>
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</tr>
<tr>
<td>Kolesnikov, Alexander</td>
<td>Moscow State University, Russia</td>
<td><a href="mailto:aak330@yandex.ru">aak330@yandex.ru</a></td>
</tr>
<tr>
<td>Kostygov, Alexei</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:kostygov@gmail.com">kostygov@gmail.com</a></td>
</tr>
<tr>
<td>Kraeva, Natalia</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:luzikhina@gmail.com">luzikhina@gmail.com</a></td>
</tr>
<tr>
<td>Lukeš, Julius</td>
<td>Institute of Parasitology, Czech Republic</td>
<td><a href="mailto:jula@paru.cas.cz">jula@paru.cas.cz</a></td>
</tr>
<tr>
<td>Lun, Zhao-Rong</td>
<td>Sun Yat-Sen University, P. R. China</td>
<td><a href="mailto:lsslzr@mail.sysu.edu.cn">lsslzr@mail.sysu.edu.cn</a></td>
</tr>
<tr>
<td>Morales, Jorge</td>
<td>Charles University in Prague, Czech Republic</td>
<td><a href="mailto:jorge.morales@hhu.de">jorge.morales@hhu.de</a></td>
</tr>
<tr>
<td>Navarro, Miguel</td>
<td>Instituto de Parasitología y Biomedicina, Spain</td>
<td><a href="mailto:miguel.navarro@ipb.csic.es">miguel.navarro@ipb.csic.es</a></td>
</tr>
<tr>
<td>Nenarokova, Anna</td>
<td>Institute of Parasitology, Czech Republic</td>
<td><a href="mailto:a.nenarokova@gmail.com">a.nenarokova@gmail.com</a></td>
</tr>
<tr>
<td>Opperdoes, Fred</td>
<td>de Duve Institute, Belgium</td>
<td><a href="mailto:fred.opperdoes@uclouvain.be">fred.opperdoes@uclouvain.be</a></td>
</tr>
<tr>
<td>Pánek, Tomáš</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:mistrpanek@seznam.cz">mistrpanek@seznam.cz</a></td>
</tr>
<tr>
<td>Paris, Zdeněk</td>
<td>Institute of Parasitology, Czech Republic</td>
<td><a href="mailto:parda@paru.cas.cz">parda@paru.cas.cz</a></td>
</tr>
<tr>
<td>Peters, Andrew</td>
<td>Charles Sturt University, Australia</td>
<td><a href="mailto:apeters@csu.edu.au">apeters@csu.edu.au</a></td>
</tr>
<tr>
<td>Podešová, Lucie</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:podesvoval@email.cz">podesvoval@email.cz</a></td>
</tr>
<tr>
<td>Polanská, Nikola</td>
<td>Charles University in Prague, Czech Republic</td>
<td><a href="mailto:sadlovaj@natur.cuni.cz">sadlovaj@natur.cuni.cz</a></td>
</tr>
<tr>
<td>Rádrová, Jana</td>
<td>Charles University in Prague, Czech Republic</td>
<td><a href="mailto:radrova@natur.cuni.cz">radrova@natur.cuni.cz</a></td>
</tr>
<tr>
<td>Schönian, Gabriele</td>
<td>Charité Universitätsmedizin, Germany</td>
<td><a href="mailto:gabriele.schoenian@t-online.de">gabriele.schoenian@t-online.de</a></td>
</tr>
<tr>
<td>Skalický, Tomáš</td>
<td>Institute of Parasitology, Czech Republic</td>
<td><a href="mailto:skalicky@paru.cas.cz">skalicky@paru.cas.cz</a></td>
</tr>
<tr>
<td>Spodareva, Viktoriia</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:spodareva@gmail.com">spodareva@gmail.com</a></td>
</tr>
<tr>
<td>Stark, Olivia</td>
<td>Charité Universitätsmedizin, Germany</td>
<td><a href="mailto:olivia.stark03@googlemail.com">olivia.stark03@googlemail.com</a></td>
</tr>
<tr>
<td>Svobodová, Milena</td>
<td>Charles University in Prague, Czech Republic</td>
<td><a href="mailto:milena@natur.cuni.cz">milena@natur.cuni.cz</a></td>
</tr>
<tr>
<td>Ševčík, Jan</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:jan.sevcik@osu.cz">jan.sevcik@osu.cz</a></td>
</tr>
<tr>
<td>Tashyreva, Darina</td>
<td>Institute of Parasitology, Czech Republic</td>
<td><a href="mailto:tashyreva@butbn.cas.cz">tashyreva@butbn.cas.cz</a></td>
</tr>
<tr>
<td>Votýpka, Jan</td>
<td>Charles University in Prague, Czech Republic</td>
<td><a href="mailto:vapid@natur.cuni.cz">vapid@natur.cuni.cz</a></td>
</tr>
<tr>
<td>Yurchenko, Tatiana</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:tatiana.yurchenko@osu.cz">tatiana.yurchenko@osu.cz</a></td>
</tr>
<tr>
<td>Yurchenko, Vyacheslav</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:vyacheslav.yurchenko@osu.cz">vyacheslav.yurchenko@osu.cz</a></td>
</tr>
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<td>Name</td>
<td>Institution</td>
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<tr>
<td>Záhonová, Kristína</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:kristina.zahonova@osu.cz">kristina.zahonova@osu.cz</a></td>
</tr>
<tr>
<td>Zidková, Lenka</td>
<td>Charles University in Prague, Czech Republic</td>
<td><a href="mailto:murfar@seznam.cz">murfar@seznam.cz</a></td>
</tr>
<tr>
<td>Zimmer, Sara</td>
<td>University of Minnesota, USA</td>
<td><a href="mailto:szimmer3@d.umn.edu">szimmer3@d.umn.edu</a></td>
</tr>
<tr>
<td>Žihala, David</td>
<td>University of Ostrava, Czech Republic</td>
<td><a href="mailto:zihaladavid@gmail.com">zihaladavid@gmail.com</a></td>
</tr>
</tbody>
</table>
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