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**ABSTRACTS**



# **SESSION I**

**Taxonomy, systematics and evolution of  
parasites**



## A preliminary biological and molecular characterization of *Babesia* sp. in the Massive Sinai, Egypt

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Babesiosis is an emerging zoonotic disease on all inhabited continents and various wildlife species may act as reservoir hosts for zoonotic *Babesia* species. The primary vectors of *Babesia* are ixodid ticks, with the majority of zoonotic species being transmitted by species of the genus *Ixodes*. Species of *Babesia* vary in their infectivity, virulence and human pathogenicity.

The aim of our study was to compare the prevalence of *Babesia* infections in four rodent communities inhabiting separated dry mountain valleys (wadies) in Sinai, Egypt. Rodents (n=1161) were trapped in four wadies in Sinai mountains during four scientific expeditions carried out in August and September in years 2000, 2004, 2008 and 2012. *Babesia* infections were detected on the basis of microscopical observation of Giemsa-stained blood smears and by the use of molecular techniques – amplification of *Babesia/Theileria* 18S rRNA gene fragment (in years 2004–2012).

The dominant rodent species was the spiny mouse *Acomys dimidiatus*, (n=834), the overall prevalence of *Babesia* infection was 3.2%. Additionally, Wagner's gerbil *Dipodillus dasyurus* (n=109) was sampled and the prevalence of *Babesia* was the highest in this species (24.7%). The prevalence fluctuated between the years of the study, however the percentage of infected gerbils remained high, especially in Wadi Gebel. Preliminary molecular characterization revealed that *Babesia* isolates derived from *D. dasyurus* were not related to *B. microti* and cannot be identified as any known *Babesia* species. Hence, further studies on these isolates are needed to determine the biological and molecular features of this new species.

The study was funded by the National Science Centre (NCN) grant OPUS 2011/03/B/NZ6/02090.

## Morphometry and internal morphology of *Codonobdella truncata* Grube, 1872

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*Codonobdella truncata* (Clitellata: Hirudinida: Piscicolidae) is an endemic species in Lake Baikal. The specific environmental requirements of the leech do not allow it to spread beyond its borders. The species occurs at a depth of 10 m to 1100 m, however, it can be probably be found more often in deeper parts of the reservoir. The leech parasite occurs primarily on amphipods, but also it is encountered on fish.

The aim of this study was the morphometric analysis of *C. truncata* as well as to obtain a detailed description of the structure of its digestive and reproductive systems. Biometrics and internal morphology were analyzed for 98 and 65 individuals of *C. truncata*, respectively.

The average length and weight of the analyzed leeches were 11.9 mm and 24.8 mg, respectively. A thorough analysis of the structure of the digestive and reproductive systems confirmed and significantly supplemented the incomplete information provided on this subject in the literature. The study showed no differences in the structure in the analyzed individuals, and repeatability in the construction of their digestive system. Furthermore, it was demonstrated that the seminal vesicles in 34% of the analyzed individuals of *C. truncata* are located at a half of the ovisacs length. The ejaculatory ducts may be straight or looped, and in 46% and 43% individuals they were looped to a medium or low degree, respectively. In the female reproductive system, the ovisacs are protuberant and elongated, and are often twisted together. Their posterior ends reach the first pair of testisacs in 69% of examined individuals.

The results of the study have added new important data to the description of *C. truncata*, which enriches the diagnosis of the leech species.



## **Systemic approach of the areas of description, classification and phylogenetic reconstruction**

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Systemic approach in science allows to use the connection between methods of systematics and phylogenetics. Thus, areas of description, classification and phylogenetic reconstruction become dependent one to another.

Deep changes in the methodology, theory and practice on systematics, that have supervened for last forty years, eventuated to distinguish three essential directions of the systematics: genealogic (eclectic), phonetic and phylogenetic (cladistics). These directions are associated with different philosophies and try to create the theoretical frames, which were lacking in the systematic until now.

This approach is constructed on the only one base – the concept of the organismal entirety, and different methods correspond to different levels of the concept.

## Phylogenetic utility of the model of a leech (Clitellata – Hirudinida) body form

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Notwithstanding the many recent phylogenetic investigations on leeches, mainly based on molecular data, the classification of those highly specialized annelids remains still uncertain.

Furthermore, there is the opinion that a widely accepted classification system needs to be supported by morphological synapomorphies, and cannot be only derived from analyses of DNA sequences.

Of the morphological features used in phylogenetic reconstruction, morphometric characters seem to be the most controversial. However, it is certain that those characters fulfill the basic cladistic condition: namely, they possess homological character states.

In this study, we demonstrate the results of parsimony analysis of 30 leech species that represent the main ecological groupings, as well as *Acanthobdella peledina* and *Branchiobdella astaci* as representatives of an outgroup. The analysis was based on two morphometric datasets derived from the model of leech body form: means used as such, and means transformed to codes by gap weighting. In both analyses, the representatives of the families Piscicolidae and Glossiphoniidae formed monophyletic clades but did not appear to be sisters. The piscicolids have shown close relations with *B. astaci*, while the glossiphoniids either created a sister clade with arhynchobdellid leeches (in the analysis based on means as such) or formed an independent phylogenetic branch sister to other Hirudinida (in the analysis based on coded means). The Hirudiniformes appeared to be a group derived within the Erpobdelliformes which is either polyphyletic (according to the analysis using means as such) or monophyletic (based on coded means). To estimate the utility of morphometric data in phylogenetic analysis, the obtained trees were confirmed using other cladograms based on morphological characters, molecular data and combined data sets. The results were very similar and the occurrence of particular monophyletic groups was recorded in each cladogram. In the present study, it was shown that morphometric characters provide additional information about the existence of monophyletic groups within the Hirudinida. Thus, the features of leech body form can be considered as a class of data appropriate for the phylogenetic reconstruction of leeches.

## Variability within species *Isthmiophora melis* (Digenea, Echinostomatinae) – molecular analysis vs. morphological analysis

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*Isthmiophora* is one genus of the subfamily Echinostomatinae and one of those with a rich taxonomical history. The validity of the genus *Isthmiophora* in relation to the genus *Euparyphium* was discussed and confirmed by Kostadinova and Gibson (2002). As a result of the revision, a new diagnosis of the genus and a list of approved species were presented. The main features distinguishing the representatives of the genus *Isthmiophora* from others of the Echinostomatinae are the following: the anterior position of the testes (the length of the post-testicular region is 30–50% that of the length of the body), the short forebody (FO=10–20%), the presence of an armed cirrus, the small head collar, the differing size of the dorsal spines (oral longer than aboral), the short uterus and large eggs (Kostadinowa and Gibson 2002). The type-species is *I. melis*, an echinostomatid with 27 collar spines reported mainly from European, Asian and American carnivores.

During helminthological investigations of rodents in different localities of Lower Silesia (SW Poland), flukes belonging to Echinostomatinae were found. These digeneans were reported only from one host i.e. the striped field mouse *Apodemus agrarius* from the ornithological reserve „Milicz Ponds”. Due to the difficulty in classifying echinostomatids from the striped field mouse, a molecular analysis based on nuclear and mitochondrial DNA data (partial sequences of genes 18S, ITS, NAD1, COX1) was performed, as well as a morphological comparative analysis between adults of *Isthmiophora* sp. from *Apodemus agrarius* and *Meles meles*, *Neovison* (= *Mustela*) *vison*, *Erinaceus europaeus*.

Based on the results of these molecular and morphometric analyses of *Isthmiophora* sp. specimens parasitizing various hosts, we comment on the diagnostic features within the Echinostomatinae subfamily and a theory of host-induced variability.

## Identification of Metastrongylidae species occurring in wild boar

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Lungworms (Nematoda, Metastrongylidae) occurring in the Suidae may be divided in two groups according to their phenotypic traits. The first group includes species whose females have provaginas in the posterior part of the body (*M. pudendotectus*, *M. asymmetricus*), whilst in the second group of species, females do not have provaginas (*M. elongatus*, *M. confusus*, *M. salmi*). The size of the provagina varies within a single species and is often characterized by a wide inter-individual variability. Similarly, there may be difficulties in identifying females from the second group. Furthermore, the dimensions of the parasites are subject to a high degree of divergence, and some species ranges overlap. Observing the terminations of copulatory spicules and the shape of the male copulatory bursa is the most authoritative means of phenotypic classification of parasites of the *Metastrongylus* genus. However, the recognition of male specimens does not conclusively identify the species of parasites present in one hosts, because their invasion has mixed nature. Hence, the molecular identification of parasites of *Metastrongylus* genus occurring in wild boars in Lower Silesia was attempted to eliminate potential errors resulting from the imperfections of the human eye.

Seven females of lungworm species from Metastrongylidae family isolated from wild boar lungs were subjected to molecular examination. The wild boar was shot in the Zlotowek Forest Inspectorate in March 2012. The genetic material of parasites was isolated using a commercial test for DNA isolation from tissue (A&A Biotechnology). A flanking sequence was amplified within the ITS-2 region, and the obtained PCR products were divided into two parts. The first part of the DNA matrix was sequenced and the results of the analysis were compared with the GenBank library. The second part of the genetic material was analyzed with the use of the RAPD method. It is a random amplification of polymorphic DNA, which relies on the random amplification of the short DNA fragments. The short primers for the study were selected based on the available literature. The DNA of different parasites species within the *Metastrongylus* genus is varied. Striped patterns characteristic of each individual species were received after electrophoresis of the PCR product on agarose gel. The affinity of the examined parasites can be assessed thanks to the similarity of the striped patterns obtained in this test. The study was conducted in two ways so that a comparison of the results will allow the costly sequencing process of genetic material of each isolated individual to be bypassed in the future.

## The evolution of mitochondrial genomes of Anisakidae (Nematoda)

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Mitochondrial genomes are considered as accumulating substitutions faster than nuclear non-coding sequences. This attribute can be used for accurate identification of cryptic species or different populations of species belonging to Anisakidae family. A life-cycle of anisakids involving several hosts increases the dispersal ability of a species, while a relatively large population size suggests a high gene flow between populations of Anisakidae worldwide. Therefore, worms belonging to the most widely-spread species, like *Anisakis simplex* s. l. and *Pseudoterranova decipiens* s. l. from different locations, could be coupled into one large panmictic population. On the other hand, a complete analysis of oceanic subpopulations and their evolutionary status is impossible when sequences with a limited number of mitochondrial genomes are used.

First goal of the study was to analyze mitochondrial genomes representing the main mitochondrial lines characteristic for each species. Second goal was to find the basic differences in the mechanisms and time of the speciation. Dataset comprising of 12 whole mitochondrial genomes of *A. simplex* s. s. and 2 genomes of *A. simplex* C, and genomes of *Pseudoterranova decipiens* and *P. bulbosa* were used to explain the evolutionary and population characteristics of species.

Results show two separated populations of *A. simplex* s. s.: Pacific and Atlantic. Pacific group is noticeably older than Atlantic one and includes few mitochondrial lines while Atlantic population is almost homogenous. The dataset including both *Anisakis* sp. and *Pseudoterranova* sp. mitochondrial sequences allow for clarify the chronology of differentiation of species within family Anisakidae. The consequence of this analysis is a good opportunity to find some information about taxonomic relationships between each species.

## What do we know about evolution of cestode egg development? Present data and perspectives.

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The Cestoda is a large group of parasitic flatworms consisting of two subclasses. Tapeworms exhibit great variation in their adult, metacestode and egg morphology. An important common feature of eucestode groups is the presence of a hexacanth larva. Cestodes differ in their types of lecithality, mode of egg formation, differentiation and embryonic development. Monozoic eucestodes, considered to be basal, have polylecithal eggs, whereas the eggs of more evolved cyclophyllideans are oligolecithal. Monozoic and other lower eucestode taxa lay eggs that are unembryonated or contain early embryos, whereas eggs of higher eucestode groups contain a pre-oncosphere or fully developed hexacanth. In monozoic and some of the lower eucestodes, the hexacanth is enclosed by an eggshell, whereas in the higher eucestodes it is surrounded by a vitelline capsule or outer coat originating from uterine secretions. Hexacanth of monozoic and lower eucestodes comprise numerous (>100) oncospherical cells and frequently contain a protonephridial excretory system, whereas in more evolved cestodes oncospherical cells are less numerous and an excretory system is absent. In monozoic and lower eucestodes, numerous vitellocytes provide material for both eggshell formation and nutrients for the developing embryo, but in more evolutionarily advanced taxa these two functions are reduced due to the development of the hexacanth *in utero*. Evident differences in the egg and hexacanth, observed between monozoic, lower and higher eucestode groups include: a progressive reduction in the number of vitellocytes per egg, the role of the vitellocyte in embryonic development, the structure and origin of the outermost layer of the egg, the number and structure of oncospherical envelopes, mainly the inner envelope that may differentiate into several layers, the morphology of the hexacanth, changes in its cell types and numbers, resulting in an evolution from oviparous to ovoviviparous type of development. Different degrees of egg lecithality, oviparity and/or ovoviviparity are closely related to the type of development and cestode life cycles.

## Advances in the biology of parasitic copepods known as sea lice (Arthropoda: Crustacea)

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The importance of parasitic copepods became evident some 20–30 years ago when the fish crowding in the intensive aquaculture operations facilitated transmission of infective stages of pathogens, in this number previously economically unimportant sea lice – the parasitic copepods of the family Caligidae. Despite the apparent importance of those pathogens of cultured fish the progress of the relevant research was very slow in the area of biology of those organisms, even though the sea lice research has been substantially financed.

The number of post-naupliar developmental stages that had hitherto been reported for two major genera of the family Caligidae – *Caligus* and *Lepeophtheirus* was different: six and eight, respectively. The complete cycle of *Caligus* included nauplius I, nauplius II, copepodid, chalimus I, chalimus II, chalimus III, chalimus IV, and adult. On the other hand in species of the genus *Lepeophtheirus* the following stages have been reported: nauplius I, nauplius II, copepodid, chalimus I, chalimus II, chalimus III, chalimus IV, preadult I, preadult II, and adult. Some researchers even tried to impose the *Lepeophtheirus* template on species of *Caligus*, reporting the existence of eight post-naupliar stages in the latter genus. This year, an international group of researchers, based on their new original studies on *Lepeophtheirus elegans*, postulated a reconciliation of the two conflicting life cycle schemes of the single family. Their interpretation, however, reconciled the two patterns in terms of the number of stages only, leaving some doubts about the nomenclature of individual stages. Our presentation is going to summarize the problem and discuss the nature of the findings reported.

## Comparison of selected parts of the genome of the tapeworm *Anoplocephala perfoliata* from different patterns of distribution of isolates

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Prevalence of *Anoplocephala perfoliata* infection has risen over past years due to changes in type of horses breeding. The diversity of *A. perfoliata* distribution observed in pilot studies indicated differences in infective influence on host health.

The present study describes two types of tapeworms distribution – scattered or clustered – that may occur in one host at one time. Genetic analysis was conducted to determine correlation between tapeworms genotype and the type of distribution. Molecular genetic analysis employed PCR of 1200-bp-long fragment of ITS1-5.8S-rRNA-ITS2 gene region. The investigated region included the whole 5.8S rRNA region and incomplete parts of ITS1 and ITS2 genes. 72 isolate samples were obtained during horse autopsies. 39 samples represented clustered form of distribution, whereas 39 were sampled from scattered distribution. To obtain significant results of DNA sequencing, the PCR products were cloned using pGEM vector. Results were analyzed with Nucleotide BLAST ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and Chromas Pro software. The final analysis comprised 47 sequences of recombinated pGEM plasmids with *A. perfoliata* DNA. Results showed multiple differences in sequenced nucleotides coding ITS1-5.8S-ITS2 region of ribosomal RNA. Differences were found in some of sequences of regions separating ITS1 and ITS2. Analysis of conservative region of 5.8S rRNA showed differences in nucleotide sequences.

Taking into consideration differences in ITS1-5.8S-ITS2 region it was possible to indicate phylogenetic differences between specimens collected from different types of distribution. However, they were not significant. No correlation between investigated DNA region and type of *A. perfoliata* distribution was found.