**Charles University in Prague**

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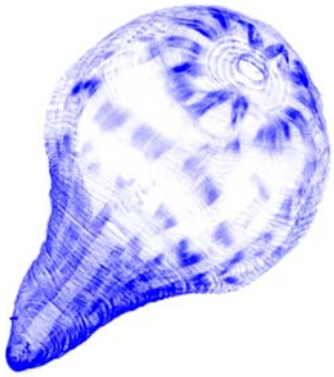
***Abstract Book***

# 3rd Workshop on Bird Schistosomes and Cercarial Dermatitis



Editors: Libor Mikeš & Petr Horák

Praha 2009

**Charles University in Prague**

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***Abstract Book***

# 3rd Workshop on Bird Schistosomes and Cercarial Dermatitis

Editors: Libor Mikeš & Petr Horák

Praha 2009

**Workshop organizers:**

*Laboratory of Helminthology, Department of Parasitology, Faculty of Science,*
*Charles University in Prague*
and
*Czech Society for Parasitology*

**Date and venue of the „3rd Workshop on Bird Schistosomes and Cercarial Dermatitis“:**

6th – 10th July 2009
Hotel Kouty, Rejčkov 20, Ledeč nad Sázavou
Czech Republic

The abstracts presented here have been published as submitted by their authors who take full responsibility for their content. No corrections have been made by the editors.

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# PROGRAMME

**MONDAY 6th JULY**

**Arrival to the hotel**

**18:00 DINNER**

**TUESDAY 7th JULY**

**7:30-8:50 BREAKFAST**

**8:55 INITIATION OF THE WORKSHOP**

**9:00-9:30 Plenary lecture**

P. Horák

Where do we go? Past and present research on bird schistosomes

*chair P. Horák*

**9:30-10:30 Taxonomy, phylogeny, biology**

S. Brant

How do we classify avian schistosome diversity and define species

J.A. Aldhoun

Mitochondrial markers as tools for bird schistosome species identification

E.S. Loker

Avian and mammalian schistosomes compared

**10:30-11:00 Coffee break**

**11:00-12:00 Avian schistosome species and their snail hosts**

K. Huňová

Taxonomy and methods of determination of the snail genus *Radix*: How many intermediate hosts are used by schistosomes of the genus *Trichobilharzia*?

D. Jouet

Avian schistosomes in France: biodiversity and host-parasite relationships

K. Skirnisson

Some facts on bird schistosomes in Iceland

**12:00-14:00 LUNCH TIME**

*chair J.A. Aldhoun*

**14:00-15:00 Diversity of bird schistosome species in specific areas**

A. Nowak

Bird schistosomes and other digenetic trematodes in the populations of freshwater snails from Brodnickie lakeland (Poland)

E.E. Kheidorova

Waterfowl schistosome invasion monitoring in the rest zone of Naroch lake in 2005-2008

E.I. Semyenova

Sequence analysis of the rRNA ITS2 from cercariae of bird schistosomes and snail hosts obtained in Russian and Belorussian water ponds

**15:00-15:30 Coffee break**

**15:30-18:00 FREE TIME**

**Petanque tournament, volleyball tournament, walking around, angling etc...**

**18:00 DINNER**

**WEDNESDAY 8th JULY**

**7:30-8:50 BREAKFAST**

*chair W. Haas*

**9:00-10:00 Host invasion by cercariae**

W. Haas

Penetration of cercariae into the living human skin: *Trichobilharzia szidati* is more successful than *Schistosoma mansoni*

K. Dolečková

Cathepsins B of the bird schistosome *Trichobilharzia regenti*

L. Mikeš

Glycocalyx composition and shedding in cercariae of *Trichobilharzia* spp.

**10:00-10:30 Coffee break**

**10:30-11:10 Pathology and immunity in vertebrates**

L. Lichtenbergová

The pathogenic effect of *Trichobilharzia regenti* migration on mouse nervous tissue

M. Kašný

Identification and characterization of dominant antigens of the bird schistosome *Trichobilharzia regenti*

**11:10-11:50 Distribution and control of cercarial dermatitis**

H.D. Blankespoor

Distribution and control of swimmer's itch in North America

C. Hörweg

Cercarial dermatitis in Austria – where do we stand in 2009?

**12:00-14:00 LUNCH TIME**

**14:00 Departure to local family brewery "Bernard" in Humpolec**

**18:00 DINNER**

**19:00 Open air grill party - smoked pork rump (and vegetables for veggies)**

**THURSDAY 9th JULY**

**7:30-9:00 BREAKFAST**

*chair C.M. Adema*

**10:00-10:40 Interactions with snail hosts**

V. Skála

*Radix* spp.: immune reactions of the snails against bird schistosome larvae – current status and perspectives

E. Žbikowska

Thermal preferences of *Planorbarius corneus* L. individuals naturally infected by *Bilharziella polonica* (Kowalewski, 1895)

**10:40-11:10 Coffee break**

**11:10-11:50 Ontogeny**

M. Chanová

The biology of bird schistosome schistosomula

J. Bulantová

*Trichobilharzia regenti*: Changes of body wall musculature during the development from miracidium to adult worm

**12:00-13:30 LUNCH TIME**

**13:30-17:00**

**Laboratory practices**

- determination of snails and cercariae
- examination of duck CNS for *Trichobilharzia regenti* developmental stages

**... IN THE CASE OF COMMON INTEREST - PANEL DISCUSSION ON CURRENT STATUS, FUTURE PERSPECTIVES AND PROBLEMS OF BIRD SCHISTOSOME RESEARCH AND CERCARIAL DERMATITIS CONTROL**

**18:00 DINNER**

**FRIDAY 10th July**

**BREAKFAST AND DEPARTURE**

# ABSTRACTS

# WHERE DO WE GO? PAST AND PRESENT RESEARCH ON BIRD SCHISTOSOMES

Horák P

*Department of Parasitology, Charles University in Prague, Prague, Czech Republic*

Bird schistosomes are well known as causative agent of cercarial dermatitis, a re-emerging disease of the present day. Therefore, control of the agent/disease was in focus, and several promising results were achiewed: a few methods for snail eradication in particular water bodies, warning systems in some areas, molecular tools for parasite identification in water samples, formulation of cream/lotion with schistosomicidal effect, etc.

On the other hand, bird schistosomes, and namely the genus *Trichobilharzia* as the most studied representative, are organisms with intriguing life cycles and host-parasite relationships. First of all, it is a rich group of morphologically similar species where only some developmental stages can be used for determination purposes. Molecular taxonomy and phylogeny play, therefore, a crucial role in recent descriptions of genera and species. It has been thought these parasites possess a narrow specificity towards their snail vectors, but this view needs a reappraisal due to new molecular analyses. Other data might also contribute to solve host-specificity issues, e.g., chemoorientation of miracidia, immune reactions of snails and immune evasion of schistosome larvae. Unfortunately, interpretation of such data is complicated by taxonomic confusion within some snail groups (e.g. within the genus *Radix*).

Bird schistosomes are pathogenic to their hosts, influencing physiology, behavior, etc. At the level of intermediate hosts, a thorough analysis of parasite effect on snail immunity, reproduction and growth has been made with the model *Lymnaea stagnalis*-*Trichobilharzia ocellata* (= *T. szidati*). Thinking about vertebrates, bird schistosomes can attack birds and mammals. In the latter case, the parasites are not necessarily trapped in the skin and can cause damage to different tissues/organs, but they do not mature to adults. With regard to this, the discovery of neuropathogenicity of *T. regenti* was a shock to us. To know more about parasite strategies in vertebrates at the molecular level, new sophisticated tools are available, allowing characterization of (a) chemical stimuli serving for parasite orientation, (b) parasite antigens and immune reactions triggered by them, (c) parasite cysteine peptidases playing a role in penetration, migration and digestion, etc.

Current intensive communication among labs/scientists having diverse expertise and interest makes our knowledge of bird schistosomes more complex; the 3rd Workshop on Bird Schistosomes and Cercarial Dermatitis certainly represents a symbol of this cooperation.

## HOW DO WE CLASSIFY AVIAN SCHISTOSOME DIVERSITY AND DEFINE SPECIES?

Brant SV and Loker ES

*Department of Biology, Center for Evolutionary and Theoretical Immunology and Museum of Southwestern Biology, Division of Parasites, University of New Mexico, Albuquerque, NM 87131, USA*

In the last decade, adoption of molecular methods in parasitology has greatly facilitated the discovery of cryptic diversity, including even fragments of worms. In many cases, these patterns of DNA relationships have provided dramatic new insights into biogeography, host use, and understanding of morphological transitions. Avian schistosomes exemplify this trend; as more snail and bird hosts are examined, more new lineages are found. Although an impressive molecular database of avian schistosome samples has accumulated over the last decade, there is still no consensus on how to use this information in conjunction with other information to define a species. How do we reconcile our DNA discoveries, which bring with them concrete insights about hsot use and biogeography, with past studies? Essential tasks for identification, other than DNA, include defining morphology and host use. While we appear presently to lack a single defining feature of a species, with results from DNA studies, we have reliable sequence information to benchmark other useful features that can be used in a key to help guide species identifications. Unlike other parasite groups, geographic isolation may not play a significant role in the diversification of avian schistosomes. The biological and ecological characteristics of migratory birds and ubiquitous distribution of snails together should facilitate interspecific host transfers and, over time, lead to non-specific host-parasite associations. While this does occur, the diversity that has been uncovered is extensive suggesting other isolation mechanisms are at play and that a new framework to incorporate this diversity is needed. Presented here is a review of biological features that define avian schistosomes within a phylogenetic framework. Features such as morphology of males, cercariae, eggs, host and habitat within hosts, and biology of the host, with a focus on *Trichobilharzia* and *Gigantobilharzia* will be discussed.

# MITOCHONDRIAL MARKERS AS TOOLS FOR BIRD SCHISTOSOME SPECIES IDENTIFICATION

Aldhoun JA[1], Webster BL[2], Littlewood DTJ[2]

[1]*Charles University in Prague, Department of Parasitology, Prague, Czech Republic;*
[2]*Department of Zoology, The Natural History Museum, London, United Kingdom*

Mitochondrial (mt) genomes of two common bird schistosome species and causative agents of swimmer's itch, *Trichobilharzia regenti* and *T. szidati*, were used to search for markers capable of quick species identification. As conservation of alignable positions proved to be important for primer design, the mt genome of *Schistosoma japonicum* was included in the alignment. Three regions were selected: cox1, cytb and rrnL-rrnS. For each of them, a set of primers giving products approximately 600 bp long was designed. The primer pairs were tested on samples belonging to eight bird schistosome species. PCR products obtained were sequenced using the same primers. PCR using cox1 primers was not successful in most cases, and the quality of the resulting sequences was poor. Both cytb and rrnL-rrnS primers worked well in PCR amplifications, and the sequences obtained by direct sequencing were of high quality. Whereas partial cytb sequences of different bird schistosome species were nearly identical, rrnL-rrnS region showed sufficient interspecific variability and more extensive sequencing was carried out. Phylogenetic analysis based on partial rrnL-rrnS sequences of 29 samples corresponded with the results based on nuclear ITS region sequences. As the rrnL-rrnS fragment is considerably shorter than the ITS region (~600 bp versus 1200-1800 bp) the new primers will enable quick and simplified identification of bird schistosomes.
This project was supported by the Czech Science Foundation (Grant No.206/07/P092), by the Ministry of Education, Youth and Sports of the Czech Republic (Grants No. MSM0021620828 and No. MSM LC06009).

## AVIAN AND MAMMALIAN SCHISTOSOMES COMPARED

Loker ES and Brant SV

*Department of Biology, Center for Evolutionary and Theoretical Immunology and Museum of Southwestern Biology, Division of Parasites, University of New Mexico, Albuquerque, NM 87131, USA*

Avian schistosomes are more numerous in genera and species than mammalian schistosomes, and it is probable there are many more avian than mammalian species left to discover. Avian schistosomes are more diverse in terms of aquatic habitats occupied, both marine and freshwater, and although some mammalian schistosomes are essentially amphibious because of the habits of their snail hosts, some avian species develop in high intertidal snails that are rarely immersed in water. Avian schistosomes occur over a broader latitudinal and geographical range and on every continent except Antarctica. Mammalian schistosomes do not occur in Australia and are rare in Europe, especially Western Europe. Avian species inhabit nasal mucosae or intestinal veins, and at least one species is an arterial specialist. Most mammalian species colonize intestinal veins, though at least one has colonized the nasal chamber, one is a urinary system specialist, and one has tendencies to colonize the arteries. Mammalian schistosomes inhabit definitive hosts with a far greater range in body size and longevity (elephants to mice) than avian species, and at least one mammalian species can inhabit a broad diversity of mammals of varying size. Avian schistosomes have a greater range in adult body form, from typically dimorphic schistosomes to slender thread-like worms with a minimum of dimorphism, to robust worms with more fluke-like shapes with little dimorphism. Adults of some avian schistosome have lost suckers, and in general are more morphologically plastic than their mammalian counterparts. With respect to snails, mammalian schistosomes colonize freshwater caenogastropods of a single family, and planorbids and lymnaeids. Avian schistosomes colonize ampullariids, valvatids, marine and freshwater caenogastropods, opistohobranchs, planorbids, lymnaeids, physids and chilinids, and exhibit several instances of dramatic host switches in snails. They have secondarily colonized marine snails and habitats. Some species of mammalian schistosomes have cercariae with eyespots, whereas all avian schistosome cercariae do. Phylogenetically, avian schistosomes range from basal to derived species. Very little is known about the breeding biology and life histories of avian schistosomes, particularly if they live for long times as the adults of some mammalian schistosomes do.

# TAXONOMY AND METHODS OF DETERMINATION OF THE SNAIL GENUS *RADIX*: HOW MANY INTERMEDIATE HOSTS ARE USED BY SCHISTOSOMES OF THE GENUS *TRICHOBILHARZIA*?

Huňová K[1)], Hampl V[1)], Kuběna A[2)], Vostrý M[1)] and Horák P[1)]

[1)]*Department of Parasitology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-12844, Prague 2, Czech Republic*
[2)]*Department of Philosophy and History of Science, Faculty of Science, Charles University in Prague, Viničná 7, CZ-12844, Prague 2, Czech Republic*

Snails of the genus *Radix* (family Lymnaeidae) are the intermediate hosts of bird schistosomes of the genus *Trichobilharzia*; infections by fasciolid flukes, *Fascioloides magna* and *Fasciola hepatica*, have also been recorded. Unfortunately, species determination within the genus *Radix* is rather problematic and unresolved. Different approaches/criteria for determination of snail species have been used in the past, especially shell morphology, anatomy of reproductive system and DNA analyses. These data, however, have been obtained separately by different authors and do not bring consistent conclusions. As a consequence, confusion in snail determination exists, and the intermediate host specificity of trematodes attacking these snails remains questionable

In our study we used and compared the above mentioned criteria for snails collected in the Czech Republic and Iceland. We dissected snails from 43 localities and characterized morphology of their reproductive system. Four groups of *Radix* snails were determined –*R. lagotis*, *R. labiata*, *R. auricularia* and *Radix* sp. The snails differed in: 1) location of *bursa copulatrix* and its duct in respect of *corpus pyriforme* and 2) the shape of bursa and the length of its duct. Dissections were followed by analysis of the ITS2 area of rDNA and determination according to shell morphology (height and width of the shell and aperture). Four species were distinguished – *R. lagotis*, *R. auricularia*, *R. labiata* and *R. peregra* according to Bargues et al. (2001). DNA analysis showed that some specimens morphologically determined as *R. lagotis* belong to *R. labiata* based on ITS2 sequences. This implies that morphology of the reproductive system is not reliable for distinction between *R. lagotis* and *R. labiata*. Moreover, morphometric statistical analysis (e.g. Multivariate general linear model, Post Hoc tests, ) of shells from the same individuals showed, that also shell morphology cannot be used for determination of particular *Radix* species. Therefore, current keys for field identification of *Radix* snails are of limited use.

Laboratory isolates of *R. labiata* and *R. lagotis* (DNA determination) were used for infection experiments with *Trichobilharzia regenti*. The main question was: Is the susceptibility of *R. labiata* and *R. lagotis* to the infection by this bird schistosome similar? *T. regenti* was able to develop in both snail species; 89 – 91 % of the infected snails shed cercariae. Thus, it has been shown, that the intermediate host specificity of *T. regenti* is not as strict as we supposed.

# AVIAN SCHISTOSOMES IN FRANCE: BIODIVERSITY AND HOST-PARASITE RELATIONSHIPS

Joutet D and Ferté H

*JE 2533 – USC AFSSA « VECPAR » UFR de Pharmacie, 51096 Reims, France*

Increasing of waterfowl populations (migratory and sedentary) and resurgence of human touristic activities on various ponds and lakes have promoted the emergence or re-emergence of cercarial dermatitis in France. On request of local authorities, some epidemiological surveys have been established to seek, understand and identify the different potential agents: parasites, final and intermediate hosts.

Snails were collected around places where Human cercarial Dermatitis (HCD) cases had been usually recorded, and cercarial emergence was tested. At the same time, water birds of these areas (found dead or killed during hunting period) were autopsied to determine the presence of nasal and visceral parasites. Among theses species, we selected ocellate furcocercariae, eggs and adults of Trematodes belonging to the avian schistosomes group, and characterized them by molecular biology.

Sequencing of the D2 domain and internal transcribed spacer (ITS-1 and ITS-2) of the ribosomal DNA (rDNA) was used for the identification of parasites (furcocercariae, eggs and adult worms) from naturally infected hosts. Among haplotypes isolated in France, some of them were found to be identical with taxa of *T. franki*, *T. regenti*, *T. szidati*, *Bilharziella polonica* and *Dendritobilharzia pulverulenta*, now considered as potential agents of HCD in Europe. Several other haplotypes, different from sequences available in the GenBank database, appeared to be new species. They were isolated from adults and eggs from Mute swans and Grey-lag geese and cercariae isolated from *Radix peregra*. Some markers (D2 or ITS-2) seems to be informative at specific level, while and the ITS-1 appears to be a good populational marker.

The concept of specificity in the relationships between Trematodes and snails is also discussed, following the observation of cercariae with several different haplotypes (corresponding with different species of *Trichobilharzia* as *T. regenti* or *T. franki*), emitted by only one species of snail (*Radix peregra*) present on the same site.

# SOME FACTS ON BIRD SCHISTOSOMES IN ICELAND

Skirnisson K

[1]*Institute for Experimental Pathology, University of Iceland, Keldur, IS 112, Reykjavík, Iceland*

In the last decade, swimmer's itch (SI) has repeatedly occurred in people that have been wading or bathing in ponds or lakes in Iceland where water birds and snails are abundant. Some of the affected sites were warmed by geothermal activity, and others were not. A search for the causative agent of SI, ocellate furcocercariae that have been found in Iceland only in *Radix peregra* snails, revealed an average infection prevalence of 1.4% (n = 12,432). Locally, infection rates commonly exceeded 6%, the highest value observed being 24.5%. A search for adult schistosomes in visceral organs and the nasal cavities of 110 water birds belonging to the orders Gaviiformes, Podicipediformes and Anseriformes revealed eggs, miracidia or adult stages of at least seven previously identifiable schistosome species in four anseriform bird species. A previously unknown species of schistosome, *Allobilharzia visceralis,* was detected in whooper swans (*Cygnus cygnus*)*,* and classified in a new genus. In mallards (*Anas platyrhynchos*) a nasal *Trichobilharzia* sp. and the visceral schistosome *T. franki* were identified. In red breasted merganser (*Mergus serrator*), distinct egg types belonging to two species of the genus *Trichobilharzia* have been found. In grey-lag goose (*Anser anser*) two different egg types were also found - a large *Trichobilharzia* sp. and small eggs of a *Dendritobilharzia* sp.. Additionally, unidentified cercariae, probably belonging to a previously undescribed genus were detected in *R. peregra* in Óslandsstjörn. Taken together, the data obtained by morphological examination of eggs and recent DNA sequencing results, indicate that at least eight species of bird schistosomes occur in Iceland. In recent months, some additional results were obtained by the examination of the local, non-migrating grey-lag goose population in Reykjavík. These data will be presented by D. Jouet during this 3rd workshop on bird schistosomes.

Special thanks are due to all the assistants and co-workers on the project, especially, however, Libuse Kolářová and Jitka A. Aldhoun in Prague and Damien Jouet and Hubert Ferté in Reims in France.

# BIRD SCHISTOSOMES AND OTHER DIGENETIC TREMATODES IN THE POPULATIONS OF FRESHWATER SNAILS FROM BRODNICKIE LAKELAND (POLAND)

Nowak A and Żbikowska E

*Nicolaus Copernicus University in Toruń, Poland*

Bird schistosomes are a group of dioecious flatworms, with complicated life cycle connected with two host – water snails (intermediate) and – waterfowl (definitive). Cercariae of these parasites, which have furcate tail and two pigmented eye spots, can accidentally penetrate into human skin causing cercarial dermatitis. This is the reason why the environmental studies concerning the occurence of these parasites in freshwater snail populations should be conducted in a great many water habitats being used as swimming holes.

The aim of our work was to determine the species composition of digenetic trematodes and the prevalence of naturally infected molluscs, with special regard to invasion of bird schistosomes. The study was carried out in selected lakes of Brodnickie Lakeland (Kujawsko-Pomorskie Province, Poland). Samples of freshwater snails were collected by hand and using a net in the littoral zone of these reservoirs from May to September 2008.

A total of 5548 studied snails belong to six families of pulmonate and prosobranch molluscs: *Lymnaeidae* (5 species), *Planorbidae* (6), *Physidae* (2) as well as *Viviparidae, Hydrobiidae* and *Neritidae* (represented by one species each). The most common species were *Lymnaea stagnalis* (1386 individuals), *Stagnicola palustris* (578), *Radix auricularia* (567), *Radix balthica* (445), *Planorbarius corneus* (1009) and *Viviparus contectus* (1229). The entire extensity of infection by larval trematodes varied from 18.4 % to 51.4 %, depending on the studied area. When compared against those figures, the prevalence of bird schistosomes amounted to less than 1 %. The *Trichobilharzia* genus was found in seven lakes (out of nine investigated) in the following species: *S. palustris* (prevalence 0.5 %, 1 individual with *Trichobilharzia* for 207 specimens infected by other trematodes), *L. stagnalis* (2.8 %; 12/430), *R. auricularia* (2.9 %; 5/172) and *R. balthica* (4.5 %; 5/111). *Bilharziella polonica* was found only in two lakes in *P. corneus* (0.6 %; 2 /313).

The results obtained during the research indicate that the prevalence of bird schistosomes in Brodnickie Lakeland is at a low level in relation to the total prevalence of digenetic trematodes. However, such low extensity of infection does not protect bathers in lakes or scientists collecting samples from freshwater habitats against 'swimmer's itch'. It was shown that one snail can release even up to six thousand cercariae (Żbikowska, 2004; Parasitol Res 92: 30-35).

# WATERFOWL SCHISTOSOME INVASION MONITORING IN THE REST ZONE OF NAROCH LAKE IN 2005-2008

Kheidorova EE and Bychkova EI

*Laboratory of Parasitology, State Scientific and Production Amalgamation „The Scientific and Practical Center for Bioresources", Minsk, Republic of Belarus*

For the purpose of control of cercarial dermatitis incidence waterfowl schistosome invasion monitoring has been held in the rest zone of Naroch Lake since 2005. 321 birds of 12 species of waterfowl were investigated on Naroch Lake in the period 2005-2008. 7 bird species were established to belong to carriers of schistosome invasion: *Anas platyrhynchos*, *A. penelope, A. crecca, A. querquedula, Aythya fuligula*, *A. ferina* and *Podiceps cristatus*. Parasites were not found in all the investigated representatives of *Fulica atra, Larus ridibundus, L. canus, Sterna hirundo, Cygnus olor*. Since 2005 in Naroch population of the duck there is a tendency towards decreasing of schistosome abundance index and invasion intensity smoothly by 2,2 times (from 12,9 to 5,95 and from 18,4 to 8,5 parasites per bird correspondingly) and of the maximum of parasites in one infected bird by nearly 3 times (from 122 to 41). Though percentage of infected individuals in this species population was on the equal level (within 70 %) during the given period. Amoung the investigated *Aythya fuligula* invasion percentage and parasite abundance index quartered approximately (from 100 % to 25 % and from 8,5 to 2 parasites per bird correspondingly). Decrease of waterfowl invasion by trematodes of the Schistosomatidae family is probably due to dehelmintization having been held in Naroch Lake since 2005. The four-year comparative analysis of invasion rates of *Anas platyrhynchos* and *Aythya fuligula* showed that duck infection by the Schistosomatidae trematodes exceeds all invasion indexs of *Aythya fuligula*. It should be noted that the maximal number of parasites in one infected duck exceeds the same level of *Aythya fuligula* by almost 10 times while percentages of infected individuals in the populations of the both species are 61,9 and 71,6 correspondingly. This fact witnesses a high adaptation level and survival rate of bird schistosomes at ducks that makes them basic nature source of invasion agents of cercarial dermatitis in Naroch Lake.

# SEQUENCE ANALYSIS OF THE RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER (ITS2) FROM CERCARIAE OF BIRD SCHISTOSOMES AND SNAIL HOSTS OBTAINED IN RUSSIAN AND BELORUSSIAN WATER PONDS.

Chrisanfova GG[1], Lopatkin AA[1], Vasyliev VA[1], Mischenkov VV[2], Zhukova TV[2], Voronin MV[3], Be'er SA[3], Zazornova OP[3], Vodyanitskaya SN[4], Yurlova NI[4] and Semyenova SK[1].

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The nucleotide sequences of the second internal transcribed spacer (ITS2) of ribosomal DNA have been observed among several taxonomical groups of invertebrate, and sequencing of this region is increasingly being applied as a diagnostic tool to identify the species of digeneans and mollusks. In this study we sequenced the ITS2 regions both from larval stage (cercariae) of bird schistosomes and their intermediate host (snails) populations. Infected snails collection (*Radix* spp., *Lymneae* spp., *Planorbarius corneus*) were recovered from 12 Russian (Moscow and Novosibirsk region, n=20) and Belorussian (Naroch Lake, n=50) water ponds. The newly obtained and known sequences of bird schistosomes and snails from Europe downloaded from the GenBank database were used. Total DNA was extracted from the feet of snails and from individual cercaria or pools of 10-15 cercariae. PCR was carried out according to Dvořák et al. (2002) and Ferte et al. (2005). Phylogenetic analyses were performed with PAUP (ver.4). Based on our study four recently recognized schistosome species (*Bilharziella polonica, T. szidati, T. franki* and *T. regenti*) and six snail species (*P. corneus, L. stagnalis, Lymneae (Stagnicola) palustris, R. auricularia, R. lagotis, R. ampla*) were distinguished. *B. polonica* was found exclusively in *P. corneus* obtained from Naroch Lake. Only *T. szidati* parasitized on *L. stagnalis* and *S. palustris* was detected in Novosibirsk region. At least three parasite species were recovered from different Moscow ponds - *T. szidati* (in *L. stagnalis*), *T. franki* (in *R. auricularia*) and *T. regenti* (in *R. lagotis*). The 25 isolates of the new *Trichobolharzia* species preliminary named as *T. sp.* var. *narochanica* were isolated from the snail *R. ampla* in Naroch Lake. The taxonomic position of studied isolates as well as the biogeography and epidemiological values were discussed.

# PENETRATION OF CERCARIAE INTO THE LIVING HUMAN SKIN: *TRICHOBILHARZIA SZIDATI* IS MORE SUCCESSFUL THAN *SCHISTOSOMA MANSONI*

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In a former study we had analysed the behaviour of *Trichobilharzia szidati* cercariae when penetrating the living human skin*. Now we used similar methods to study the cercarial skin invasion of the human parasite *Schistosoma mansoni*. We placed gamma-irradiated and nonirradiated cercariae onto the living human skin and timed their behaviour. In order to obtain complete data for 53 individuals, we needed 133 trials with a total of 715 cercariae. The results showed that skin invasion of *T. szidati* was much more efficient than that of *S. mansoni*. *T. szidati* cercariae crept shorter on the skin after attachment until they started penetration movements (median of 8 s (0-80 s), *S. mansoni* 43 s (15 s-6.58 min). Within this shorter exploratory phase, they found more suitable entry sites than *S. mansoni*: no *T. szidati* cercaria penetrated into the smooth skin surface (*S. mansoni* 22%), 84% penetrated into wrinkles (*S. mansoni* 74%), and 16% into hair follicles (*S. mansoni* 4%). For full entry the *T. szidati* cercariae needed on average 4.0 min (1.38-13.34 min), *S. mansoni* cercariae 6.58 min (1.57-13.13 min), the fastest cercaria entered the skin within 83 s, *S. mansoni* 94 s. All *T. szidati* cercariae shed their tails within 0-105 s after onset of penetration movements, whereas 60% of the *S. mansoni* cercariae had the tails still attached when the bodies disappeared in the skin.

The faster invasion of *T. szidati* cercariae may result from the more sophisticated host-finding mechanisms of this species. *T. szidati* cercariae respond to more host-specific cues than *S. mansoni* cercariae (for attachment and enduring contact: ceramides and cholesterol, *S. mansoni* arginine), and when *T. szidati* cercariae use similar host cues, they respond with higher sensitivity (warmth for attachment, fatty acids for penetration, furthermore arginine, arginine containing peptides and glucose for migration in tissues). A faster entry might also result from different enzymes used in skin penetration (probably cathepsin B-like cysteine peptidases in *T. szidati* and the serine peptidase elastase in *S. mansoni*).

Interestingly, penetration of *S. mansoni* cercariae did not release a cercarial dermatitis as immediate skin response, although the senior author had 3 histories of acute *S. mansoni* schistosomiasis and responded sensitively to invasion of *T. szidati* cercariae. A cercarial dermatitis against *S. mansoni* developed only after a sensitization period of 19 days and 8 infection series.

*Haas W, van de Roemer A (1998) Invasion of the vertebrate skin by cercariae of *Trichobilharzia ocellata*: penetration processes and stimulating cues. Parasitol Res 84, 787-795

## CATHEPSINS B IN THE BIRD SCHISTOSOME *TRICHOBILHARZIA REGENTI*

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*Trichobilharzia regenti* is a nasal avian schistosome, closely related to medically important human species of the genus *Schistosoma*. The aquatic free-swimming larvae, cercariae, are the causative agents of cercarial dermatitis in humans. The species is unusual by its neurotropic mode of migration in vertebrate hosts.

Parasite-derived proteases often play a crucial role as the key components of many tasks, necessary for the parasite survival, including protein processing, tissue penetration, immune evasion and nutrition uptake. Moreover, some of them have been identified as attractive targets for chemotherapy or potent selective diagnostic markers.

In this work we investigate the temporal distribution of cysteine peptidases - cathepsin B1.1 and cathepsin B2 - in several developmental stages of *T. regenti* by real-time polymerase chain reaction (PCR). The analysis showed that the gene expression of cathepsin B1.1 is considerably more developmentally regulated than cathepsin B2 during the life cycle of *T. regenti*. Moreover, in both cathepsins, the highest level of transcription was detected in maturing and adult stages of the parasite.

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## GLYCOCALYX COMPOSITION AND SHEDDING IN CERCARIAE OF *TRICHOBILHARZIA* SPP.

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Cercarial stages of trematodes posses a relatively thick glycocalyx serving as a protective coat in the environment. Its composition is known mostly from the species parasitizing humans, namely *Schistosoma mansoni*. Saccharide molecules are bound to lipids or proteins on the membrane of cercarial tegument. During the penetration into a vertebrate host, the glycolalyx is actively shed by the cercariae as it is highly immunogenic and may be also attacked shortly after penetration by components of host's innate immunity. Only limited information exists about the molecules involved in glycocalyx shedding – mainly proteolytic enzymes from cercarial penetration glands have been suspected.

Glycocalyx of trichobilharziae has been studied by a few authors, mainly its saccharide composition by lectin-binding techniques, less by electron microscopy or immunological methods.

In our experiments, we tested a set of lectins to characterize differences in glycocalyx saccharide composition among cercariae of three species of the genus *Trichobilharzia – T. szidati*, *T. regenti* and *T. franki* – in order to evaluate the usefullness of the lectin binding technique in species differentiation. Unfortunately, the lectin binding pattern was almost identical for all three species tested.

In the experiments with induction of penetration gland emptying and staining of the gland content we observed strong binding of the postacetabular content to the surface of cercariae of *T. szidati*. However, they were able to shed the material from their surface within a short time. Testing the reactivity of cercariae with fluorescent lectins before and after induction of gland emptying showed that the surface undergoes significant changes – after the reaction with the gland content it does not bind selected lectins anymore. The visualization of glycocalyx shedding will be presented and discussed in context of molecules putatively involved in this process (including peptidases contained in cercarial penetration glands). Immunogenicity of cercarial glycocalyx for specific definitive hosts (ducks) will be also documented.

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# THE PATHOGENIC EFFECT OF *TRICHOBILHARZIA REGENTI* MIGRATION ON MOUSE NERVOUS TISSUE

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*Trichobilharzia regenti*, parasite of freshwater birds, belongs into a small group of schistosomes with adults located in the nasal area of the definitive hosts. The route of *T. regenti* migration in definitive host is not common among Trematodes. The migration starts in the skin where cercariae transform to schistosomula. Then the flukes invade peripheral nerves and continue through CNS to the nasal area.

Cercariae of *T. regenti* can accidentally penetrate into the skin of mammals (including humans). Nevertheless, the parasites are not able to complete their development in the noncompatible host and die at certain point after infection. In humans, the skin phase of infection is manifested by cercarial dermatitis, an inflammatory reaction which leads to an elimination of the parasites. However, experiments with mice showed that not all the schistosomula are trapped and destroyed, the parasites are able to escape from the skin and migrate through the nervous system. In mammalian nervous tissue, the parasites cause pathological changes analogous to those observed in birds. In certain cases, the infections are manifested by various neuromotor symptoms.

In order to find out the pathological effect of *T. regenti* on mouse nervous tissue, mice (Balb/c) were infected by *T. regenti* cercariae. The nervous tissue was dissected at various intervals after infection and examined by histological and immunohistochemical methods. According to microscopical observation, CNS infection started 2 days p.i. Progression of the infection invoked an inflammatory reaction with a formation of granulomatous lesions around the schistosomula and increased proliferation of astrocytes in grey and white matter of the spinal cord. Presence of the parasite triggered expression of MHC-II molecules on the surface of astrocytes and also increased expression of adhesive molecules ICAM-1 on endothelium which facilitated influx of the immune cells, CD4+ lymphocytes and macrophages.

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# IDENTIFICATION AND CHARACTERIZATION OF DOMINANT ANTIGENS OF THE BIRD SCHISTOSOME *TRICHOBILHARZIA REGENTI*

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*Trichobilharzia regenti* is a bird schistosome related to human pathogen *Schistosoma mansoni*. Although *T. regenti* can finish its life cycle only in specific waterfowl host *Anas platyrhynchos*, their larvae - cercariae (similarly as those of *S. mansoni*) are able to penetrate, transform and migrate as schistosomules in non-specific hosts (e.g. mouse, man), too. The penetration can be accompanied, analogically to specific hosts, by an early type of hypersensitive reaction known as cercarial dermatitis ("swimmers´ itch"), which is followed by a late phase of cutaneous inflammation. In our study we monitored the level of antibody response of mice (C57BL/6 strain) infected by *T. regenti*. Mice were infected 4 times with the cercariae and tail blood was collected prior to each infection and subsequently at day 10, 20, 30, 40, 60 and 120 after the last infection. The serum antibodies reactions with mix *T. regenti* antigens - cercarial homogenate (TrH), excretory/secretory products of cercariae (TrE/S) and cysteine peptidases – cathepsins B1/B2 (TrCB1/B2) were tested by ELISA and Western blot. Employing ELISA**,** the negligible IgG2b/IgG2a response (markers of Th1 immune response) was detected and dominant IgG1 response (associated with Th2 immune response) with TrH and TrE/S antigens was determined. By using Western blot with TrH and TrE/S the strong reaction of IgG1 and IgE at the area 25 and 34 kDa was revealed. The protein bands in these areas (25/34 kDa) were identified by mass spectrometry analysis (MALDI TOF-TOF, followed by *de novo* sequencing) as triose-phosphate isomerase (25 kDa) and glyceraldehyde 3-phosphate dehydrogenase (34 kDa).

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## DISTRIBUTION AND CONTROL OF SWIMMER'S ITCH IN NORTH AMERICA

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Based on responses to a questionnaire on the website, "swimmersitch.org" it appears that 98% of the swimmer's itch cases occur in the northern tier of states and in Canada. States reporting the most cases include Michigan, Wisconsin, Minnesota and Washington. British Columbia and Ontario have the most cases in Canada. The above distribution correlates well with the geographical distribution of the snail intermediate host, *Stagnicola* and the diving ducks belonging to the genus *Mergus*. Control efforts include the capture of the common mergansers, treating them with Praziquantel and relocating them to a suitable habitat on the Great Lakes where the snail intermediate hosts are not located.

## CERCARIAL DERMATITIS IN AUSTRIA – WHERE DO WE STAND IN 2009?

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Cercarial dermatitis is known in Austria since 1971, with regular occurrences nearly every summer. Nevertheless, the knowledge on the spectrum and biology of both parasites and snails is still fragmentary. Some data have been published on cases of swimmer's itch and the occurrence of schistosomatid cercariae in Austria, but these papers mostly list case records and offer no detailed biological, ecological or taxonomic analyses. At the moment two species, i.e. *Trichobilharzia szidati* and *Bilharziella polonica*, have been proved to occur in Austria. Concerning the snails, it has been found that not only species of *Radix*, *Lymnaea* and *Stagnicola*, but also *Gyraulus parvus* and *Aplexa hypnorum* may act as intermediate hosts.

A website was created [http://www.helminths.at/zd/badederm.html] both to provide information for the public and to get data from affected persons. The main aim was the documentation of the occurrences to get an idea on potential locations for further investigations. Therefore a questionnaire was designed and people were asked for the following parameters: personal data, information about the waters, activity in the water and details about the dermatitis itself.

A total of 34 questionnaires showed that most cases were reported from so-called quarry ponds where people have weekend homes. Waterfowl were recorded from the waters in every case. With one exception, an itching dermatitis occurred, but less than one third of those affected people sought consultation.

In fact questionnaires are tools easy to provide, but difficult to interpret, because one has to rely on the data transmitted. The results of the questionnaires helped to identify a new group of people, holders of private natural swimming ponds, which seem to be at risk as well.

## *RADIX* SPP.: IMMUNE REACTIONS OF THE SNAILS AGAINST BIRD SCHISTOSOME LARVAE: CURRENT STATUS AND PERSPECTIVES

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Bird schistosomes are obligatory dixenic parasites using, like many other trematodes, snails as the intermediate hosts in their life cycle. Namely, the genus *Radix* Montfort, 1810 (family Lymnaeidae) represents vector snail of the nasal bird schistosome, *Trichobilharzia regenti* Horák, Kolářová et Dvořák, 1998 (family Schistosomatidae). However, not all species of the genus *Radix* can serve as intermediate hosts of *T. regenti*, and it is believed that the parasite-intermediate host combinations are species-specific. Such specificity is given (at least partly) by the snail defense system that is able to kill schistosomes in non-specific snails; hemocytes probably play the most important function in this process.

In our experiments, the snails of *Radix lagotis* were infected by miracidia of *T. regenti* and histological evaluation of infected individuals was performed at intervals 1, 2, 3, 5, 12, 16, 20, 36, 44, 60 and 92 hours post infection (p.i.). Remarkable infiltration of hemocytes around schistosome larvae was observed from 2 to 20 hours p.i. Then, the number of hemocytes gradually decreased till 44 hours p.i.; later no hemocytes being attracted by schistosomes were present. This confirms our hypothesis that *R. lagotis* as a susceptible intermediate host does not mount an effective response and its cellular reactions can be suppressed by parasites.

Among mechanisms of snail immunosuppression parasite surface saccharides might play an important role: they could disrupt transmembrane signal pathways of hemocytes with subsequent effect on hemocyte phagocytosis, spreading, migration and other functions (Plows et al. 2005). This hypothesis will also be tested in the combination *Radix lagotis*-*Trichobilharzia regenti*. Last but not least, the presence/absence of other defense factors (e.g. lectins, fibrinogen-related proteins, etc.) in *R. lagotis* will be studied.

# THERMAL PREFERENCES OF *PLANORBARIUS CORNEUS* L. INDIVIDUALS NATURALLY INFECTED BY *BILHARZIELLA POLONICA* (KOWALEWSKI, 1895).

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The objective of this study was to determine whether individuals of *Planorbarius corneus* naturally infected with *Bilharziella polonica* larvae had different thermal preferences than non-infected (control) ones, and to examine the influence of constant temperature on host survival.

During the first part of the study the snails were placed in an oblong thermal gradient adapted to the automatic registration of freshwater invertebrates' behavior. The six experimental groups of *P. corneus* were tested - three groups were kept in induced wintering conditions (three months in +4$^{o}$C): 1. individuals with emerging *B. polonica* cercariae, 2. snails with *B. polonica* sporocysts without emerging cercariae, 3. non-infected (control) animals, and three adequate groups which were in active state (studied in the period of one week after the collection of samples in the lakes). The two out of three studied factors had an influence on thermal behavior of snails: the infection and the cercariae liberating. No difference between thermal behavior of active and wintering snails was noted.

During the second part of the study three experimental groups of snails were kept in two different thermal conditions: +22$^{o}$C and +27$^{o}$C. The first-one was the temperature approximated to that chosen by hosts with emerging cercariae, and the second-one was preferred by other animals under study. In higher temperature both groups of infected snails (with emerging and without emerging cercariae) lived shorter than non-infected ones.

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## THE BIOLOGY OF BIRD SCHISTOSOME SCHISTOSOMULA

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Schistosomulum is the first stage developing in definitive host body, affecting various body parts and in the case of bird schistosomes present in host tissues for longest period. The aim of the presentation is to summarize recent knowledge of bird schistosomula migration, development and pathogenic impact on host tissues.

Schistosomulum is formed by transformation of cercaria in the host skin at the time of penetration. The process is preceded by cercarial tail detachment and includes emptying of penetration glands and extensive surface changes.

Transformed schistosomula migrate towards the target organ. Depending on the species schistosomula migrate via the circulatory system or nervous tissues and the migration is directed either to intestinal or nasal area (visceral or nasal species, respectively). Specific migratory pattern for lung passage of *T. szidati* and migratory route of *T. regenti* through the nervous system, unique among schistosomes and including intra- and extra vascular location, are obligatory for bird schistosomula.

Schistosomula feed on host red blood cells, except for *T. regenti* ingesting either red blood cells or particles of nervous tissue in certain phases of migration.

Pathological consequences of bird schistosome infections caused by schistosomula are of hight significance (contrary to mammalian schistosomes with low pathogenic impact of migrating larvae). Schistosomula of the neurotropic *T. regenti* cause degenerative changes of the nervous tissues, and a leg paralysis and balance disorders develop. Lung stage schistosomula of visceral species cause an inflammatory reaction with infiltrates formed around the blood vessels and in the gas-exchange tissues, followed by pneumonia, oedema and hemorrhages development.

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## *TRICHOBILHARZIA REGENTI*: CHANGES OF BODY WALL MUSCULATURE DURING THE DEVELOPMENT FROM MIRACIDIUM TO ADULT WORM

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The musculature of bird schistosomes changes with respect to the developmental stage and its mode of life. The most useful techniques for muscles observation in human schistosomes were confocal microscopy with FITC-conjugated phalloidin for labeling of filamentous actin, and transmission electron microscopy (TEM) for characterization of ultrastructure.

The same techniques were applied in order to characterize an avian schistosome, *Trichobilharzia regenti*. The species exhibits a unique life strategy among schistosomes, because of having affinity to the nervous system of birds and experimental mammals. Obviously, several systems/organs of parasite body play an essential role in invasion of and migration within host body, e.g. secretory system, nervous system, muscles and tegument. Therefore, filling the gap in our knowledge, all developmental stages of *T. regenti* (model organism), from miracidium to adult worm, were characterized with respect to body wall musculature.

The body wall musculature of all stages comprises two coherent layers of muscles: circular and longitudinal. Cercariae, schistosomula and adult worms have additional groups of muscles - radial muscles (connecting the inner layer of subtegumental muscles with internal organs) and diagonal muscle fibers which cross each other at the angle of 120° and create sparse net. Circular and longitudinal muscles, having compact appearance in migrating early mother and daughter sporocysts, gradually enlarge their distance and turn into sparse, grid like structure of stationarily developing sporocysts.

Description of bird schistosome musculature, and its similarity to that of human schistosomes, can contribute to formulation of a generalized view on the muscle structure and function of medically important trematodes; such knowledge might be of high value e.g. in the development of new drugs operating at the level of neuro-muscular synapses.

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