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PREFACE

A long-lived lake can be extant (ancient lake) or fossil (paleolake). Ancient lakes typically have continuously existed since before the last glacial period (i.e., ~120,000 years ago) or even since before the Pleistocene some 1.8 million years ago. Lakes such as Baikal, Tanganyika, Malawi, Biwa, and Ohrid, have long been recognized as centers of biodiversity and during the past decades, ancient lakes have emerged as an important topic in the fields of systematics, paleontology, evolutionary biology, and conservational biology. Given this growing interest, an international workshop on "Speciation in Ancient Lakes" was held in Belgium in March 1993, and it was decided to establish SIAL as an international organization in order to stimulate interactions between scientists working on ancient lakes (see <http://www.sial-online.org>). It was also decided to change the name of the organization to "Species in Ancient Lakes." However, for consistency, the regular meetings of SIAL are continued to be referred to as "Speciation in Ancient Lakes."

Since then, SIAL regularly organizes international meetings at institutions with a visible and long-standing history of ancient lake research, often directly located at one of the known ancient lakes. These meetings serve as an important basis for exchanging latest and state of the art information on ancient lake research, to discuss new research approaches and projects, and to get students and young scientists interested in ancient lake research.

Keeping up with this tradition, SIAL5 is taking place at one of the world's best recognized ancient lakes – Lake Ohrid – jointly organized by the Hydrobiological Institute in Ohrid and the Justus Liebig University in Giessen.

Lake Ohrid is probably 2–5 million years old and harbours approximately 1,200 native species. In terms of endemic biodiversity, the lake is with 212 known endemic species and a surface area of 358 km² one of the most diverse lakes in the world, taking surface area into account.

Of course, SIAL5 does not exclusively deal with Lake Ohrid but with a variety of old lakes in the world, both extant and fossil, and this proceeding volume includes 86 abstracts of oral presentations and posters accepted for presentation at SIAL5. These presentations cover diverse topics ranging from micro-speciation to conservation issues and ecology of ancient lakes.

The organizers hope that SIAL5 will further deepen the awareness of old lakes in the scientific community and particularly among students, and that it stimulates further research on those lakes that ultimately may lead to a better understanding of their past, present, and future.

We would like to thank the other members of the organizing committee for their hard work. These are, in alphabetical order, Andrew S. Cohen (U.S.A.), George Coulter (New Zealand), Matthias Glaubrecht (Germany), Doug Haffner (Canada), Hiroya Kawanabe (Japan), Goce Kostoski (Macedonia), Zdravko Krstanovski (Macedonia), Koen Martens (Belgium), Frank Riedel (Germany), Isa Schön (Belgium), Christian Sturmbauer (Austria), Oleg Timoshkin (Russia), and Risto Väinölä (Finland). Special thanks go to Christian Albrecht (Germany), the secretary of SIAL5, who did a splendid job in coordinating the scientific part of SIAL5. Finally we would like to thank the employees of the Hydrobiological Institute and the people of Ohrid for their support and hospitality.

Sasho Trajanovski & Thomas Wilke

TRACKING BIOLOGICAL INVASIONS INTO ANCIENT LAKES: *PHYSA ACUTA* (GASTROPODA: HYGROPHILA) ON THE BALKANS

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Biological invasions, particularly of gastropod species have become a major problem in disturbed landscapes. However, very few recent gastropod invasions of ancient lakes have been demonstrated, e.g. in Lake Malawi and in Lake Titicaca. The globally invasive *Physa acuta* is native to North America and is established in many regions of Asia, Australia, and Africa. It was an early invader in Europe, i.e., at the beginning of the 19th century or earlier, and has achieved a nearly pan-European distribution within the last two centuries. *Physa acuta* is known from all Balkan countries, however, no comprehensive study on the presence of the invasive species in the natural and particularly in the ancient and potentially ancient lakes of the Balkans exists. During ongoing studies on the evolution and diversity of molluscs in European ancient lakes, we had the opportunity to survey selected natural lakes in the Balkans. Extensive field data obtained from 2003 to 2009 with repeated samplings in a total of 21 lakes was analyzed and the literature was reviewed. The purpose of this study is a) to review and evaluate the presence of *P. acuta* in the major Balkan natural lakes and b) to discuss potential reasons accounting for the successful establishment of *P. acuta* or its absence in particular lakes. Given the often high morphological plasticity of the shell of physid gastropods, the species identity was ascertained by morphological and molecular methods. Starting with the

first recording in Lake Dojran (Macedonia) in 1985, *P. acuta* is now present in at least 14 lakes. For the first time, a thriving population of *P. acuta* was discovered in ancient Lake Ohrid in May 2009. The population was found at the Ohrid Bay in close proximity to the channel leading to the Hydrobiological Institute Ohrid.

Considering that the current environmental situation in almost all Balkan lakes includes increasing eutrophication and loss of habitat, the successful invasion of *P. acuta* might be directly linked to human activities. As yet it is impossible to predict what competitive impact the invader might have on native and particularly on the endemic gastropod faunas of Balkan lakes. Only concerted international plans of action can perhaps improve the environmental situation in the lakes concerned and thus potentially slow down the further spread of the invasive *Physa acuta*.

DETECTION OF MICROCYSTIN SYNTHETASE GENES IN THE DIFFERENT WATER BASINS OF RUSSIA

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Cyanobacteria bloom in lakes and reservoirs and the deterioration of their water quality induced by it, has become a serious problem for many countries. Most common and well-known cyanotoxins are microcystins, cyclic peptides consisting of 7 amino acids. Microcystin intoxication

affects the liver. The microcystin synthetase gene cluster (*mcy*) has a modular structure, where each module is responsible for the synthesis of specific enzymes.

This work is aimed at identifying toxic cyanobacteria in samples collected during the summers 2005-2008 from Lake Baikal, Lake Kotokel, water reservoirs of East Siberia and Kurshsky Bay (Baltic Sea). Toxic cyanobacteria were detected by two genetic markers: *mcyE* gene responsible for the transition of glutaminic acid into an active D-form and *mcyA* gene coding specific peptide synthetase.

We found species capable of producing microcystin in samples from all of the water bodies studied. These are the representatives of *Microcystis*, *Anabaena*, *Planktothrix* genera, *M. aeruginosa* being most abundant among them. In Lake Baikal and Irkutsk Water reservoir the concentration of the species was low. *M. aeruginosa*, *A. flos-aquae* proliferated in Ust-Ilimsk, Bratsk, Krasnoyarsk Water reservoirs and Kurshsky Bay, a hypereutrophic freshwater lagoon. Mass growth of these species was also observed in Lake Kotokel, where several episodes of intoxication in humans were registered.

PCR-analysis revealed no microcystin synthetase genes in Baikal and Irkutsk reservoir, whereas in other water bodies PCR-test showed positive results. Phylogenetic analysis revealed that most of the sequences obtained are highly homologous with many *M. aeruginosa* isolates from other water bodies. Three clones from Kurshsky Bay showed 99% homology with that of *M. viridis*, and *mcyA* gene from Bratsk reservoir belong to *Anabaena* sp.

Thus, the data obtained enables us to conclude that the water bodies of various origin which were studied, geographical location and trophic status house potentially toxic representatives of widespread *Microcystis* and *Anabaena* species.

INTERANNUAL VARIATIONS IN THE SEASONAL DYNAMICS OF PHYTOPLANKTON FROM LAKE BAIKAL

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Spatiotemporal variations are constantly registered in the plankton of Lake Baikal: perennial cyclic fluctuations and intra-annual seasonal phenomena which remain important long-term research.

Analysis of the data collected between 2005 and 2008 enabled the estimation of the intra- and inter-annual variations of the phytoplankton species composition and its quantitative indices in Baikal.

In some years, a closer look at the phytoplankton revealed significant deviations in the algal composition, as well as marked changes in their abundance and biomass. It was found that the phytoplankton sampled in the pelagial during 2005-2006, which were the years of low productivity, are characterized by a higher abundance of green colonial algae, various autotrophic flagellates. *Chroomonas* sp., *Rhodomonas pusilla* and *Cryptomonas reflexa*, were the most abundant cryptophytes. During these years, average long-term abundance and biomass values in a 0-25 m layer amounted to 121 thou. cells/l and 80 mg/m³, respectively.

2007 was a highly productive year, whereas 2008 showed average productivity, mainly due to "bloom" of *Synedra acus* diatoms. In 2007, *Stephanodisus meyeri*, *Aulacoseira islandica* and *A. baicalensis* were also productive, and in 2008 spring phytoplankton was dominated by *S. acus*, its percentage comprising over 90% of the total amount. The last

“bloom” of *S. acus* was observed in 2001 and 2003 in Southern Baikal. As previously noted, it may occur in substantial amounts for two years. In autumn 2008, maximum abundance of *Cyclotella minuta* was registered, but during preceding years its number was observed to be under 20 thou. cells/l. It is known that the peak of *Cyclotella minuta* does not coincide with the “Melosire” years, its maximum being found only after two “Synedra” years. In 2007-2008, average perennial quantitative values in the 0-25 m were: abundance – 558 thou. cells/l, biomass – 597 mg/m³. Examination of the results of long-term phytoplankton observations provided a basis for the assessment of Baikal ecosystem sustainability and an enhancement of the reliability of the forecasting.

**HOLOCENE MORPHOSPACE EXPANSION OF *BELLAMYA*
GASTROPODS FROM LAKE MALAWI AS POTENTIAL EVIDENCE
FOR PUNCTUATED MORPHOLOGICAL CHANGE**

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A relationship between species diversification and morphological divergence is proposed by several major evolutionary concepts, but only few studies have attempted to disentangle how both processes interact. Much work, certainly in palaeontology, requires at least some insights into this hypothetical correlation to allow confidential interpretation of morphological changes in an evolutionary context. Here we study

changes in the shell morphology of the Malawian gastropod genus *Bellamyia* that underwent molecular diversification from a single ancestral lineage in Lake Malawi since Middle-Pleistocene times. Since mid-Holocene times, a 3.6-5.1 times morphospace expansion from a homogeneous ancestral morphotype is evidenced. Taphonomic information suggests this rapid expansion to represent an evolutionary event rather than an artefact created by sampling bias. Our data suggest morphological diversification in sympatrically occurring *Bellamyia* gastropods to postdate the onset of molecular diversification, and maybe even to postdate speciation, for post-zygotic reproductive isolation is reported to have been completed at least by some of the recognised *Bellamyia* species from Lake Malawi. The observed burst in morphological change may conform to morphological punctuations much like the sense proclaimed by punctuated equilibrium evolution.

THE DIVERSITY OF THE T4-TYPE BACTERIOPHAGES IN THE LAKE BAIKAL

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Bacteriophages are the most abundant biological entities in the biosphere and play a key role in global biogeochemical cycling. T4-type bacteriophages are an important component in the *Myoviridae* family particularly from an ecological viewpoint. Recently a set of degenerate PCR primers for phage T4 g23 gene was designed which encodes the

major capsid protein in all of the T4-type phages. These primers were used to amplify g23-related sequences from diverse marine environments and from paddy field agroecosystems. The present study was aimed at elucidating the molecular diversity of T4-type bacteriophages in Lake Baikal by targeting g23 genes of T4-type bacteriophages.

In June 2008 water samples were collected from two stations in the northern and southern parts of Lake Baikal from the lake surface to 50 m depth. The water samples were sequentially filtered through polycarbonate filters (Millipore) with a diameter of 1.2, 0.45 and 0.2 μm . The filtered subsamples (100 ml) were then filtered through 0.02 μm Anodisc aluminum oxide membrane filters (Whatman). DNAs were extracted from 0.02 μm filters with DNA-sorb kit (InterLabService) and amplified with degenerate primers targeting the g23 gene. The obtained and purified g23 gene PCR products were cloned. In total 41 clones were sequenced and translated into a deduced amino acid sequence. Phylogenetic analysis of g23 clones clearly demonstrated that some of Baikal sequences belonged to the group of the T4-type cyanophages, the ExoT-evens. The major portion of the sequences obtained in this study was closely related to previously described subgroups of uncultured T4-phages from marine and paddy field environments. This part of the sequences formed several separate clusters.

Thus, our studies have revealed a high diversity of the T4-type myoviruses in Lake Baikal and have shown the presence of g23 genes both similar to those retrieved from various environments and exclusively specific to freshwater ecosystems.

MICROBIAL COMMUNITY OF DEEPWATER SPONGES IN LAKE BAIKAL

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Microbial community, associated with deepwater sponges in Lake Baikal, has been studied using molecular biology methods. Sponges were collected at depths of 200 to 900 m by the deep diving manned submersible "MIR". During diving of 2008-2009 we have collected 10 samples of sponges from different areas of Lake Baikal.

Total DNA from all samples was phenol extracted and investigated. PCR with universal eubacterial primers was conducted, also crenarchaeal and methanotrophic primers were used. All products were cloned and sequenced. Analysis of fragment gene of 16S rRNA reveal followed groups of microorganismas: alphaproteobacteria (*Azospirillum*, *Methylocella*), betaproteobacteria, gammaproteobacteria (Methylococcales), actinobacteria (Acidimicrobiales), Bacteroidetes (Flavobacteria), Planctomycetes .

With using group-specific primers we found out sequences of uncultivated methanotroph I type (profundal sediment of Lake Constance), uncultured bacteria from methyloph populations in an acidic forest soil, uncultured methanotrophic proteobacteria, different uncultured crenarchaeota. At the present time analyses of microbial community of deepwater sponges are being continued.

Authors acknowledge the group of company 'Metropol' for organization of the expedition with using the deep diving manned submersible "MIR".

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**THE LAKE MALAWI SCIENTIFIC DRILLING PROJECT (LMSDP):
HISTORICAL ECOLOGY OF AN ANCIENT LAKE AND LESSONS
LEARNED FOR SPECIATION STUDIES USING SCIENTIFIC DRILLING**

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In 2005, the LMSDP successfully recovered seven drill cores from two locations in L. Malawi. The longest core, ~382 meters, representing about 500-600ka, was collected from almost 600m water depth in the central part of the lake. Most detailed research to date has been on a shorter core (1C, 76m length) from this same site, which has a basal model age of ~145ka.

Core 1C has a rich fossil record, including ostracodes, insects, molluscs, fish, diatoms and other algae, pollen, and charred plant particles. In total, they demonstrate profound limnological change in L. Malawi, in terms of lake level, water chemistry, nutrients and mixing, as well as the lake's surrounding environment and climate. The older portion of this record (~135-90ka) provides evidence of a series of *megadroughts*, also evident in cores from L. Tanganyika and Bosumtwi, as well as from dune deposits across southern Africa. During the most severe of these events L. Malawi was reduced to a <100m deep, saline/alkaline lake and its surrounding watershed was converted to a semidesert. Since ~70ka, L. Malawi has been a deep and relatively freshwater lake, including during the LGM period, when lake levels were ~100-200m below modern but when conditions were much more similar to modern than during the megadroughts. Major fluctuations in Late Pleistocene lake levels appear to be linked to insolation changes in the southern tropics, correlated with eccentricity-forced precessional cycles.

Differences in the response of the three largest African lakes to aridity, and especially differential responses to both the early Late Pleistocene vs. the LGM arid intervals are useful in understanding the differences in the phylogenetic histories of their endemic species. Results from the Malawi drilling experience demonstrate the need for collecting multiple drill cores from different water depths to obtain a complete paleontological record of individual groups of endemic organisms.

GENETIC DIVERSIFICATION IN SHALLOW- AND DEEPWATER AMPHIPOD CRUSTACEANS OF LAKE BAIKAL

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The oldest lake on Earth, Baikal, persists since Early Tertiary, but developed under the influence of orographic and climatic perturbations of the past 30 Myr. Today the lake is inhabited by rich endemic fauna. Amphipod crustaceans are among the most diverse groups of animals in the lake. Some 350 known species occupy a wide variety of niches spanning from shallow littoral to deep abyssal.

It has been hypothesised that the faunal radiation was affected by environmental changes, which were especially pronounced during the Pleistocene. Particularly, the Baikal deep-water fauna was suggested to have evolved only after the cooling that enabled deep-water oxygenation. Lake level changes associated with the climatic cycles might have enhanced species fragmentation and population size changes, and more strongly for the shallow-water than for deep-water fauna. The MOLARCH project (Molecular Archives of Climatic History) aimed to explore and compare patterns of intraspecific genetic variation between different groups of Baikalian fauna, to assess the congruence in patterns of diversification and their correlation with Pleistocene environmental changes. The three main hypotheses (patterns) to be tested were: 1. Synchronic diversification events across taxa that coincide with major

environmental changes; 2. Synchronic diversification, unlinked to environmental changes; 3. No correlation across taxa.

We assessed mitochondrial COI gene sequence variation (~600 bp) in 5 genera of Baikalian amphipods with different ecological preferences: *Brandtia* (shallow-water benthic), *Dorogostaiskia* (shallow-water, sponge commensal), *Acanthogammarus* (shallow-water, nectobenthic), and *Garjajewia* and *Brachyuropus* (deep-water nectobenthic). Sequence diversity was studied at inter-species, intra-species and intrapopulation levels.

Minimum inter-species (intra-genus) divergence was about 25% (model corrected distance). Applying conventional COI rates (2–5% per Myr), intra-generic diversification would seem to have already happened before the Pleistocene climatic cycles; this is also true for the deep-water taxa. However, no congruence of intra-genus radiations was found across taxa. At the intra-species level, two contrasting patterns of diversification existed: high diversity and strong geographical structuring (allopatric diversification) in shallow-water species, vs. low diversity and no geographical structuring in deep-water species. Shallow-water fauna seems to have been subject to intensive fragmentation, while deep-water taxa remained uniform throughout species ranges. Yet there were no regular patterns of intra-population diversity to suggest similar demographic histories for different species from similar localities, or for a given species from different localities, and so there is no indication of a critical role of environment or life style as a predictor of population demography and diversity. Severe bottlenecks were inferred both for some deep-water species and for some local shallow-water populations.

POSSIBLE ROLE OF INTRON MUTATIONS IN PROMOTING OF REPRODUCTIVE ISOLATION BETWEEN SISTER SPECIES

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One of the introns of a nuclear gene coding for phosphofructokinase has been sequenced in seven species of endemic Baikalian gastropods of family Baicaliidae. The length of this sixth intron (according the structure of the longest transcript of the gene in *Drosophila melanogaster*) differs between various molluscan species. The length of this intron appeared to be almost equal in distantly related species - *Korotnewia semenkewitchi*, *Pseudobaicalia zachwatkini*, *Teratobaicalia ciliata*, *Maackia costata* and *Maackia herderiana*, so that the variation is only several base pairs because of short insertions/deletions in the nucleotide sequences, whereas two closely related species *Baicalia carinata* and *B. turiformis* have dramatically different lengths of the intron, which is substantially shorter than in the other species of the family Baicaliidae due to long deletions.

Deduced secondary structures of the intron RNA suggest that introns of each species acquire different conformations due to only few base substitutions and indels. At that location of the potential stems in the RNA structure varies even between closely related species and these distinctions consist in not great numbers of the substitutions and indels. Interestingly, the most probable conformation of intermolecular heterozygotes where one strand belongs to *B. turiformis*, and the other one is *B. carinata* (closest sister species in the study) contains

complicated hairpin structure where one of the strands becomes inverted due to distorted symmetry. This increases the likelihood of chromosomal rearrangements in the region in possible inter-specific hybrids acting as weak crossingover lock.

It is well documented in studies on fruit fly, that the intramolecular rearrangements occurring within genome may cause crossingover blocking on certain chromosome region, and therefore cause at least partial restriction of the chromosome conjugation in course meiosis in the interspecies hybrid. This increases probability of the hybrids sterility or decreased viability. If this mechanism will act in sufficiently large number of sites in the non-coding DNA, it may be regarded as a catalyst of speciation by restricting recombination and therefore enhancing reproductive barrier.

LACUSTRINE RADIATIONS IN AFRICAN *SYNODONTIS* CATFISH

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Lake Tanganyika, the oldest African rift lake, is one of the world's richest freshwater ecosystems. It differs from other water bodies in this region, in harbouring numerous unrelated endemic faunas, many of which form species flocks. As such, Lake Tanganyika offers great potential to investigate comparative systems that may help us to better understand processes responsible for clade diversification.

In comparison to the cichlid fishes of this lake, non-cichlid faunas have been largely overlooked. However, *Synodontis* catfish, which comprise a small radiation in Lake Tanganyika, make a good comparative system to cichlids due to their similar biogeographic distribution as well as being a species-rich clade. These catfish were the subject of two previous independent studies that aimed to investigate the origin and timing of this group within Lake Tanganyika, but found differing results regarding their evolutionary history. Here I present data expanding upon previous phylogenetic analyses, from the inclusion of additional taxa and characters that hopefully resolve this conflict.

Molecular dating reveals a single colonisation event for the flock, which has subsequently diversified in full lacustrine conditions. The diversification of this catfish clade is comparable with a timescale based on fossil calibrations for the cichlids in this lake, as well as *Platythelphusa* crabs. While *Synodontis* catfish have not radiated in lake Malawi, it is suggested that southern riverine species speciated in a now extinct palaeo-lake, as demonstrated in cichlid fishes from this region.

ASSESSING DIVERSITY AND EVOLUTION OF LAKE TANGANYIKA'S SPONGES USING DNA BARCODING

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The diversity, systematics and ecology of sessile invertebrates in ancient lakes, particularly sponges (Porifera), are poorly understood despite their likely pivotal ecological function as filter feeders. Sponges require careful hand-collection underwater, are often delicate to transport, and are perceived by some to be less charismatic than, for example, cichlid fish, so explaining the dearth of work on this group. This is compounded by challenges in freshwater sponge taxonomy for the non-expert due to a depauperate suite of diagnostic morphological characters. We have sought to redress this by a series of underwater surveys of sponge occurrence in the Kigoma region of Lake Tanganyika, coupled with collections for systematic study. We used a first pass morphological approach to categorize morphospecies and worked to link this with the established taxonomy. Clearly, a means of molecular identification such as DNA barcodes will accelerate the process of unambiguous species identification and reveal cryptic species.

Here, we present preliminary results on molecular barcoding approaches on freshwater sponges of Lake Tanganyika, which provides first insight into lineage diversity and morphological plasticity of this group. This is part of the Sponge Barcoding Project (www.spongebarcoding.org) which is the first worldwide barcoding project on any diploblast taxa, and covers the complete taxonomic range of Porifera. We also demonstrate that the barcoding approach not only facilitates the assessment of biodiversity, but also sheds light on evolution and radiation of sessile invertebrates in Lake Tanganyika.

SIGNALS OF DIFFERENTIAL IMPACT OF ENVIRONMENTAL CHANGES ON DEMOGRAPHIC HISTORIES OF INVERTEBRATES FROM LAKE BAIKAL

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Previous studies on genetic variation of invertebrate species from the Lake Baikal revealed variation of population dynamics presumably caused by geological events and changes in global climate. In this study we investigate how species with different ecological affinities but from the same geographical area respond to environmental processes. We sampled species with low dispersal ability, so they inhabit exactly the same geographical range. We focused on a total of four species of Gastropoda and one species of Amphipoda at the South-Western shore of the Lake Baikal. We sequenced mitochondrial COI from 114 samples of *M. herderiana*, 57 samples of *B. carinata*, 19 samples of *B. carinatocostata*, 15 samples of *B. turiformis* and 43 samples of *G. fasciatus*. Comparative analysis of mismatch distributions for the invertebrates was conducted using R library APE. We revealed that mismatch distributions for *M. herderiana* and *G. fasciatus* have similar shapes and both are smooth and unimodal, whereas *B. carinata*, *B.*

carinatocostata and *B. turriiformis* demonstrate more diversity and rather multimodal shape of the mismatch distribution. We conducted Bayesian skyline reconstruction of demographic history using BEAST v.1.4.8 and found that all investigated species undergo demographic changes approximately simultaneously. In particular, *M. herderiana* underwent a dramatic expansion. *G. fasciatus* and *B. carinata* indicate signals of population growth opposite to *B. carinatocostata* and *B. turriiformis*. The time of demographic changes was estimated at 15 000 – 30 000 years ago, assuming a COI mutation rate equal to 1-2%/MYR. This time corresponds to the last glaciation period and, moreover, paleo-reconstructions demonstrate that amount of atmospheric precipitation and diatom sedimentation in the lake was reduced for that time period. Thus, our study demonstrates that past environmental changes could have resulted in contrasting demographic histories of species inhabiting the same geographical area.

ESTABLISHMENT AND EXPANSION OF LAKE MALAWI ROCK FISH POPULATIONS AFTER A DRAMATIC LATE PLEISTOCENE LAKE LEVEL RISE

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Major environmental events that fragment populations among multiple island habitats have the potential to drive large-scale episodes of speciation and adaptive radiation. A recent palaeolimnological study of sediment cores indicated that Lake Malawi underwent major climate-driven desiccation events 75,000 - 135,000 years ago that lowered the water level to at least 580m below the present state and severely reduced surface area. After this period lake levels rose and stabilised, creating multiple discontinuous littoral rocky habitats. Here we present evidence that establishment and expansion of philopatric rock cichlid populations occurred after this rise and stabilisation of lake level. Within populations of *Pseudotropheus (Maylandia)* we found evidence of a broad lake-wide spread of ancient mitochondrial DNA lineages, together with extensive local or regional accumulation of unique derived haplotypes. Coalescent analyses revealed populations only started to expand and accumulate this genetic diversity after the lake level rise. Allopatric populations of congeneric rock cichlids of Lake Malawi often differ in morphology and male colour traits, and tend to mate assortatively. Thus we conclude that this single large-scale environmental event has been central to the generation of the spectacular rock cichlid diversity that characterises this ancient lake today.

MOLLUSCS OF THE ANCIENT PALAEO-LAKE LORENYANG, TURKANA BASIN: ANAGENESIS VS. CLADOGENESIS AND THE MIGRATION OF FAUNAS

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The Pliocene-Pleistocene transition between 2.3 and 1.5 Ma was a time of central importance for human evolution. However, the evolution of hominins is difficult to study directly in Eastern Africa; one obvious reason for this being the rarity of fossils. Climatic and environmental changes are supposed to be the driving forces of evolution and migration of hominins during that time, when the ancient palaeo-lake Lorenyang filled the Turkana Basin during most of this period. The sediments of this lake in the Koobi Fora region preserve a very rich fossil mollusc fauna. Both quality of preservation and abundance of specimens is very high, rendering this fauna most suitable for quantitative palaeontological studies. In the past, Peter Williamson studied these molluscs and interpreted the faunal changes through time as evidence for the theory of punctuated equilibrium. Although this work was debated intensively, no evidence for alternative theories based on actual field data was available to date, in particular for the so called adaptive radiation in the upper Burgi Member of the Koobi Fora Formation. We anticipate, as an alternative to cladogenesis (e.g. speciation) as suggested by Williamson, diversity and disparity of molluscs through time are the result of environmental changes in the aquatic system (anagenesis). However, the most significant faunal

change in the upper Burgi Member reflects the immigration of specimens and species, respectively, instead of the creation of new taxa. Climatic changes in the catchment area of the tributaries to Lake Lorenyang caused changes of the drainage pattern of these rivers and the associated change of the river fauna introduced to Lake Lorenyang. This hypothesis is verified both by palaeontological and geochemical data. Further analyses carried out during our ongoing project should eventually also lead to a better understanding of the palaeoecology of hominids in Eastern Africa at a time critical in our origin.

POTENTIAL ENDEMIC AMONG PROTISTS, HELMINTHS, AND CRUSTACEANS IN LAKE BIWA, JAPAN

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Since 2006, the Lake Biwa Museum has been exploring the taxonomic diversity and molecular systematics of the aquatic biota in and around this ancient lake. We have also sought additional endemic taxa in the lake beyond the 57 recognized in a recent review, while recognizing that too few other water bodies have been surveyed in Japan to demonstrate endemism conclusively. Among protists, two species represent new genera of ciliates, *Levicoleps biwae* (Colepidae) and *Apocarchesium rosettum* (Vorticellidae). The former is particularly interesting since some other genera of Colepidae have been proposed as endemics of ancient lakes. Laboratory rearing studies and gene sequencing suggest that the digenean fish parasite *Genachopsis gigi*, considered a synonym of the widespread *G. goppo* since 1995, might indeed be a Lake Biwa endemic. Of the 40 species of ostracod crustaceans now confirmed from the lake, 16 are new and possibly endemic. Of these, *Fabaeformiscandona* includes the greatest number (nine species), but they do not appear to form a species flock; the four new species of *Limnocythere* might conceivably do so, however. Contrary to these examples, none of the 47 species of chironomid midges newly recorded for Lake Biwa is either undescribed or potentially endemic.

**EMBRYONAL AND POSTEMBRYONAL DEVELOPMENT OF THE
LARVAE OF *ARCTODIAPTOMUS STEINDACHNERI*, RICHARD, 1897
(CRUSTACEA: CALANOIDA) FROM LAKE OHRID**

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A. steindachneri is an euplanktonic species. It has a limited distribution and it is to be found in the lakes Ohrid, Prespa and Ioannina. In the Lake Ohrid it is permanently present and there are two known periods, i.e. period of intensive reproduction and period of delayed development which is characteristic for postembryonal development (copepodid). Their postembryonal development is rather complex and it lasts for several months. They spend a substantial part of their lives as larvae and that is why they contribute a lot to the total biomass of the zooplankton, which is quite important for the food chains, i.e. for the biological transformation of the energy in the lake ecosystems. In this work an attempt is made for determination of the time required for postembryonal development of the larvae (naupliar and copepodid) in natural conditions – the lake and in laboratory conditions. The duration of the development stadiums in natural conditions is determined from the research of their dynamics in the lake. The adult females with eggs are evidenced in the period from June to September. They attain higher numeric values during June and August. The naupliar stadiums have two greater picks: mid-June and mid-August. The larvae of the copepodid development phase achieve the maximum numeric values in September. In the analyzed material, the larvae from the fourth and fifth copepodid stadium are predominant. The time interval between the evidenced picks of the ovigerous females (June and August), as well as the picks of the larvae development stadiums is estimated at approximately two months. The adult form are absent during the autumn, winter and spring period. The population comprises relatively high number of individuals which are in the development phase IV and V copepodid. In the water they are in active state, but the development stagnates. The reason for this phenomenon is certainly as a result of the rather complex set of factors which are constantly present in the water and which are repeatedly changing. One of the main reasons is

presumably the quite low temperatures during that period of the year. With the increase of the temperature of the water during May they finish the ontogenetic development and continue their life cycle. Since the duration of the embryonal and postembryonal development of the larvae depends on the temperature, in the laboratory circumstances, the duration of the postembryonal development had been analyzed at the following temperatures: 10⁰C, 14⁰C, 18⁰C and 22⁰C. According to the results it is concluded that the duration of the embryonal development of the eggs is in inverse correlation to the temperature. The duration of the postembryonal development of the larvae depends on the temperature of the water. The changes in the duration and the speed of the development of the larvae are greater between the temperatures of 14⁰C and 18⁰C. The deviations of the data attained in the laboratory and the regression line are greater for the same temperature levels. From the four different temperature levels at which the larvae are fostered in laboratory conditions, most similar results with the quantitative data from the analysis from the lake are noted at the temperature of the water at 18⁰C. At this temperature the duration of the development of the eggs and the larvae is on average 70 days, while in the lake is approximately two months. In accordance to the monitoring of the dynamics of *A. steindachneri*, we concluded that the reproduction process is occurring during the summer period at high temperatures of the water, while the naupliar and copepodid larvae from this species are to be found in highest abundance in the water at depths of 30m to 10m where during that period the temperature of the water is in between 15⁰C and 20⁰C. By taking into consideration the attained knowledge, we can conclude that the temperature amplitude of 15⁰C to 20⁰C is to be considered as optimal for the development of *A. steindachneri*.

**DO GEOCHEMICAL PROCESSES REGULATE SPECIATION AND
DISTRIBUTION PATTERNS OF ENDEMIC SPECIES IN THE MALILI
LAKES OF SULAWESI ISLAND, INDONESIA?**

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The Malili Lakes are ancient lakes estimated to be 1 – 4 millions years old, and are the only ancient lakes that are part of a hydrological continuum. Current theory on species range extensions would predict that the advective flow among the lakes would result in a homogenization of species resulting in very similar communities/food webs. The biological assemblages of the Malili Lakes, however, reflect a distribution of species where endemic species are more commonly associated with a single lake, and this pattern has been observed for diatoms, zooplankton, shrimps, snails and fish communities. Associated with this lake to lake specific distribution of endemic species is a lack of cosmopolitan species and a failure of introduced species to flourish.

Limnological studies of the lakes have revealed distinct geochemical characteristics where iron and chrome (CrVI) enrichment has the potential to regulate primary production and also result in natural toxicological stress that has the potential to limit colonization by cosmopolitan species as well as enhance mutation rates. The lack of primary production in the lakes is associated with high levels Fe ($0.1 - 0.2 \mu\text{M L}^{-1}$) that regulate the bioavailability of nutrients such as phosphorus, and has resulted in an energetically limited food web without a top trophic level. Therefore

predation by piscivores (top – down control) has not played an important role in species interactions. In fish and snails there is strong evidence that resource partitioning in a food limited environment has resulted in the formation of distinct taxa with associated modifications of feeding structures.

Concentrations of CrVI (150 nM L^{-1}) in the water column and historic high levels of Cr in sediment cores suggest that natural toxicity has long been a characteristic of these lakes. Previous research has concluded that Cr concentrations were responsible for the failure of Cladocera to colonize these lakes. CrVI is known to be a strong mutagenic agent, and the low phosphate and sulphate concentrations observed in the lakes can potentially facilitate CrO_4^{2-} uptake. These relatively high exposures to Cr can facilitate local adaptation providing a competitive advantage to endemic species and simultaneously prevent successful colonizations by more cosmopolitan species.

LONG-LIVED LAKES AS EVOLUTIONARY ISLANDS: EXAMPLES FROM THE NEOGENE OF CENTRAL AND SOUTH-EASTERN EUROPE

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Long-lived lake faunas are in various aspects reminiscent of island faunas. Both faunas are isolated and develop high rates of endemism and

disparity. This paper provides a comprehensive discussion on the general signature of long-lived lake mollusc faunas and evolutionary trends observed in molluscs of Neogene long-lived lakes in central-eastern Europe. Diversity and disparity patterns of independent lake faunas are documented and (dis-)similarities with “classical” island faunas are considered. We collated and analysed a meta-data set on 1184 gastropod species and 202 dreissenid bivalve species from Early Miocene to Pleistocene lake systems. The data set was assembled using literature as well as museum collections. Each lake system is characterised in terms of species diversity, endemism, size and longevity. Correlations between γ -diversity, endemism rates, lake size and longevity have been investigated. Aspects of disparity are investigated using the character of maximum shell size as a proxy. For two examples, the gradual morphologic change towards gigantism is documented. In both cases this gigantism is followed by a reshaping phase due to extinction events.

There is no straightforward relationship between lake size and species richness. An imperfect correlation exists between estimated lake duration and species richness. The studied lake faunas are characterised by very high endemism ratios, low numbers or total absence of immigrant species and the development of high disparity. Species richness in the lakes is almost exclusively generated by in-situ diversification. Episodic extinctions within these lakes may have served as major resetting tools in the faunal development. A fine-scaled stratigraphic solution of fossil lake deposits is required in order to study and unravel the development of faunas in isolated lake systems. In the studied lake systems immigration is almost absent once an endemic fauna has become established. Diversity increase resulted from divergences within the lakes. There is no clear area-diversity relation in the lakes. However, the size of appropriate

habitats (excluding for example anoxic deeper zones in many lakes) may have varied dramatically through the history of such lakes. The study is a contribution to the Austrian FWF-Project P18519-B17.

GASTROPOD DIVERSITY AND DISTRIBUTION IN ANCIENT LAKE OHRID

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Ancient Lake Ohrid constitutes an outstanding hotspot of freshwater gastropod diversity in the world. To date, 73 species of gastropods are reported for the Lake Ohrid watershed with 65 of them (89%) being endemic. An extensive survey of the lake's molluscan fauna conducted between 2003 and 2008 with 270 sampled localities revealed a total number of 64 gastropod species including 56 endemic taxa (88%).

Ecological and phylogeographical analyses indicate that there is relatively little faunal exchange and overlap between Lake Ohrid and its sister lake, Lake Prespa. Within the Ohrid basin, endemism occurs at different spatial scales, ranging from species endemic to certain parts of Lake Ohrid to species endemic to the whole watershed. Cluster analyses indicate a strong bathymetric (vertical) zonation of gastropod biodiversity with the highest species richness seen in the littoral. In addition to vertical zonation, there is also some degree of horizontal zonation, mostly associated with different habitat types.

A heat map analysis of gastropod biodiversity revealed small patchy areas in the upper littoral of the south-eastern shore characterized by sublacustrine springs and diverse habitats that are home to a disproportional high amount of narrow-range endemics. However, there also is growing evidence that poorly studied regions at the northern and western shore are more diverse than previously thought and that they, at the same time, suffer comparatively higher anthropogenic pressure.

It is hoped that this and future research on the biodiversity of the lake's gastropod fauna will help protecting this unique European hotspot of biodiversity.

GASTROPOD DIVERSITY AND ENDEMISM IN THE BALKAN PRESPA AND MIKRI PRESPA LAKES

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The exact age of a given lake is often unknown, resulting in confusion about its designation as ancient lake. Moreover, a lake may have come into existence a long time ago but not have persisted continuously throughout its history. This and restricted faunal knowledge partly account for the number of unrecognized ancient lakes in the world that are outshone by their famous counterparts. The Balkan Peninsula possesses such candidate lakes that all show some degree of (gastropod) endemism. Among such lakes are the two Prespa lakes (Albania, Macedonia, Greece). Lake Prespa, located at an altitude of 853 m a.s.l. and covering 253 km² is the highest major lake in the Balkan. Subterranean karstic channels are known to be the only outflow of Lake

Prespa. The maximum depth of Lake Prespa is approximately 60 m. Lake Mikri Prespa is 47 km² in surface area, 8.4 m deep and located at 850 m a.s.l. This lake is connected to Lake Prespa. Its current water level is regulated by the outflow to Lake Prespa. Both lakes are seriously threatened by water loss and particularly eutrophication.

The malacofauna of the Prespa lakes has not been surveyed for several decades. Here we aim at assessing the current status of the gastropod fauna of the Prespa lakes using recent field survey data. The endemic mollusc species of many of the Balkan lakes were often first described only a few decades ago. We found 9 of the described 12 endemic gastropod species during our survey from 2003 to 2008 (75%). A total of 11 non-endemic species occur in the Prespa lakes. The decline and potential loss of endemic mollusc diversity in Balkan lakes is also obvious in the Prespa lakes.

Effects of human-induced environmental changes are evident for the Prespa lakes with water level loss and eutrophication (mostly due to increased phosphorous loads) being the most serious threats. These processes are particularly severe for microsnails, like most endemics, live in a small stretch of the littoral. Fast water level and/or biofilm changes have a great impact on the highly adapted communities. Changes are recognizable in the whole ecosystem as outlined, for example, by the presence of invasive fish species. These circumstances and the reported decline in endemic gastropods should trigger efforts to save this sensitive lake ecosystem. By 2009, none of the Prespa lake endemic molluscs were listed in the IUCN Red List of Threatened Species.

DISTRIBUTION AND A PUTATIVE PHYLOGENY OF THE ATYIID SHRIMPS OF LAKE TANGANYIKA

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The atyiid shrimps of Lake Tanganyika comprise an endemic flock occupying all oxygenated habitats in lake. Open water species provide a important trophic link in the pelagic ecosystem and are likely of major importance for fisheries production. Inshore species comprise *inter alia* cryptic taxa and have possible commensal relationships with shell-dwelling cichlids. Genetic analysis of mitochondrial gene CO1 and nuclear gene LSU has been done to identify genotypic distributions of *Limnocaridina tanganyika* in the southern and central sections of the lake, and have provided the first attempt to produce a phylogeny for the species flock. Population structure of *L. tanganyikae*, which is distributed widely in the littoral zone of the lake suggests a general homogeneity of genotypes, but with possible separation of populations that occur in the south west of the lake.

COMPARATIVE STUDY OF THE EVOLUTION OF SPONGES (PORIFERA) IN ANCIENT LAKES

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Freshwater sponges (Porifera; Spongillina) include 6 families. Some of them are endemic of the ancient lakes Baikal, Tanganyika, Ohrid, Malawi and Kinneret. Other families are cosmopolitan and inhabit inland waters worldwide. The taxonomy of freshwater sponges is problematic which complicates an estimation of their biodiversity. In addition evolution and phylogenetic relationships of these animals are not well known.

Freshwater sponges from lakes Baikal (family Lubomirskiidae), Biwa (family Spongillidae), Tanganyika (family Potamolepidae) and Kinneret (family Malawispongiidae) were collected and analyzed by morphology and molecular methods. Several gene regions (18S rRNA gene, COXI gene and internal transcribed spacers of rRNA) were sequenced and analyzed. The results obtained allowed the development of a molecular marker for species identification in Porifera and the study of phylogenetic relationships between endemic and cosmopolitan freshwater sponges.

Our results indicated that the endemic family Lubomirskiidae is monophyletic, showing the highest bootstrap support. The genetic

distances between Lubomirskiidae species are much smaller than those between Spongillidae species. The lower genetic distances between species of Baikalian sponges is indicative of their relatively recent radiation from a common ancestor. The evolutionary age of the family Lubomirskiidae does not exceed the geological age of Lake Baikal, which indicates an autochthonous evolution of sponges in Lake Baikal. The cosmopolitan family Spongillidae is paraphyletic in relation to other freshwater sponges. The genera *Radiospongilla* and *Eunapius* are probably monophyletic and the genus *Ephydatia* is paraphyletic and forms basal branches. Malawispongiidae of Lake Kinneret are closely related to *Ephydatia fluviatilis* that make this family polyphyletic. The family Potamolepidae is more closely related to the common ancestor of all freshwater sponges.

The molecular method developed can be useful for the identification of species of freshwater sponges. Study of species diversity of this important group of organisms will allow for the ecological monitoring of ancient lakes ecosystems.

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BACTERIAL DIVERSITY OF THE ENDEMIC FRESHWATER SPONGE *LUBOMIRSKIA BAICALENSIS*

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Aged more than 600 million years, sponges (Porifera) are the most ancient extant group of metazoans. This phylum consists of approximately 15,000 species inhabiting marine and freshwater environments. Although most sponges are found in the marine environment, an estimated 150 species populate freshwater habitats. In the oldest and deepest lake on earth, Lake Baikal, sponges formed the endemic family Lubomirskiidae, which comprises 13 species. One of the most abundant of them is a single branchy species *Lubomirskia baicalensis*, reaching lengths of up to 1.5 m. Molecular ecological studies have suggested that a majority of sponge-associated microbes are uncultured. Elucidating the genetic information of uncultivable microorganisms should provide significant insight into co-evolution between sponges and their symbionts, as well as microbial physiology and biotechnology. A number of research groups have assessed prokaryotic diversities within various marine sponge-associated bacteria by 16S rRNA gene analysis. But there is extremely little information concerning freshwater sponge bacterial communities. The aim of this study was to describe the bacterial 16S rRNA gene diversity associated with the endemic sponge *L. baicalensis*. In order to describe the diversity of uncultivable microbial communities of *L. baicalensis* 16S rRNA gene library were prepared: PCR of total DNA isolated from *L. baicalensis* tissue using 16S rRNA primers specific for bacteria yielded a band of the expected size of 1500 bp; combined PCR products were cloned into the vector pTZ57R/T (Fermrntas), yielding 160 independent clones. Seventy-five of these clones were subject to restriction fragment analysis (RFLP), resulting in 29 restriction patterns. One clone from each group was randomly chosen for sequencing. Sequence profiles obtained were submitted to the GenBank. The sequence results indicate abundant bacterial diversity in sponge *L. baicalensis*. About 40% of sponge-derived

microbial sequences were related to *Actinobacteria* (n = 11), which is a potential resource for drug research. Clones affiliated with *alpha-Proteobacteria* (n = 7), *beta-Proteobacteria* (n = 5), *Verrucomicrobia* (n = 5), *Bacteroidetes* (n = 1) were also observed. This study is the first molecular diversity study of Lake Baikal sponge-associated microbial consortia.

**FEEDING PREFERENCES AND FEEDING APPENDAGE
MORPHOLOGY OF ENDEMIC SHRIMP FROM LAKE TANGANYIKA,
EAST AFRICA**

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Lake Tanganyika, the oldest and deepest lake in Africa, is known for its remarkable species diversity and endemism. Owing to very recent speciation (lake age of 9-12 My), Lake Tanganyika's endemic species are highly suitable to study the evolutionary processes of speciation and adaptive radiation. This project focuses on evolutionary species divergence within the Tanganyikan endemic shrimp, containing the four genera *Atyella*, *Caridella*, *Limnocaridina* and *Macrobrachium* (previously *Palaemon*). The research will attempt to reveal mechanisms of diversification within the species flock, such as that of trophic partitioning, through the examination of feeding preferences and morphological adaptations towards a specific food source.

As shrimp tissue represents the consumed food sources integrated over time, Stable Isotope Analysis (SIA) was performed on the tissue of a number of shrimp species. SIA suggested a degree of trophic partitioning, providing indications of potentially specialised diets among the different shrimp species. There was, however, also considerable overlap in consumed food sources among the species, suggestive of more generalist feeding strategies. Gut content analysis of the same individuals used for the SIA will be also be done to provide independent support for the SIA results.

To reveal possible adaptations towards a feeding preference, morphological structures of the feeding appendages were studied by Scanning Electron Microscopy (SEM). Initial SEM photographs indicate considerable morphological diversity among the species studied. Combining these results with those from the study of feeding preferences may indicate whether these endemic shrimp species have specialised towards a specific diet, which may have driven speciation within the species group.

Results from both the SIA and SEM work will be examined in the context of the phylogenetic relatedness of the Tanganyikan shrimp. The phylogeny provides indications of the evolutionary background of this enigmatic group, and will help to extract the evolutionary history of the feeding appendage structures.

**EFFECTS OF LAND-WATER ECOTONE DEGRADATION ON
BIOLOGICAL DIVERSITY IN LAKE BIWA, AND ITS
CONSERVATION/RESTORATION MOVEMENTS AND PLANS BY
PEOPLE AND LOCAL GOVERNMENT**

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In the former SIAL meetings held in 2002 and 2006, the senior author explained effects of environmental degradations on biological diversity in Lake Biwa during the past 70 years, and several initiatives to combat the degradation issues by local general public as well as the passing of some byelaws and progressing measures for conservation by local and national governments. Issues included were declining water quality and invasive species, besides lake-shore ecotone problems.

In this meeting we will focus on the issues of land-water ecotone around Lake Biwa, and not only the effect of the degradation of ecotones on biological diversity but also recent initiatives and plans for their restoration in co-operation with the general public and local government: e.g., restoration of reed and aquatic-plant regions, reconstructing inner bays or lagoons, which were reclaimed as land, remaking connecting routes between the lake and paddy fields around it, etc.

We will introduce also a new scheme for ecosystem conservation/restoration and recovery of 'lake-culture complex', healthy ecosystems and human life styles, which might be expected to create until 2050 similar situations as in the later half of the 1950s.

The contributions of Lake Biwa Museum and Lake Biwa Environmental Research Institute to these issues will be briefly included in the presentation.

DIVING INTO THE UNKNOWN: SNAIL DIVERSITY AND ASSEMBLAGES IN LAKE TANGANYIKA'S SMALL-DELTA-HABITATS

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Our improving knowledge of Lake Tanganyika's endemic malacofauna has focused on the systematics, distribution and ecology of rocky substrate gastropod assemblages where extensive SCUBA sampling has provided data on spatial scales of diversity and response to anthropogenic sedimentation. In contrast, we know little of the diversity, distribution and ecology of soft-substrate gastropod assemblages nor their susceptibility to environmental change.

We sampled two paired small-stream-deltaic habitats in the Kigoma region (northern Tanzania) under contrasting levels of anthropogenic impact to document the living soft-substrate gastropod assemblages, their diversity and local spatial distributions, and any effects of increased sedimentation. Previous studies have suggested soft substrate gastropods to occur throughout the lake while rocky substrate snails are

known to have highly patchy distributions. In this study, species abundance and community composition were analyzed at three scales. 1) The smallest scale consists of 4 quadrats spaced 10m apart at 5m water depth, together comprising a site; 2) At each delta three sites were positioned with respect to the stream mouth. 3) The largest scale comprises the two streams, Kasekera (reference) and Mtanga (impacted), each draining a small, distinct watershed located along the eastern shore of the lake ~17km apart.

We found 18 species of gastropods (7-11/site). All species occur at both deltas, with the exception of one gastropod species (*Syrnolopsis carinifera*) common at Kasekera but absent from Mtanga. Bray-Curtis cluster analysis of abundance revealed a high degree of similarity of quadrats on spatial scales within a 10m distance. However, there were no patterns of sites grouping according to orientation (i.e. on-delta or off-delta) or stream system. Analysis of presence/absence data recovered no association at any scale, perhaps because of rare species. We found no pattern of faunal similarity at scales greater than 10m suggesting composition of snail assemblages is not determined solely by distance from stream mouth. Mtanga Stream has lower species richness and evenness, measured using a statistical analysis program, suggesting that anthropogenic impacts watershed specific may be affecting diversity and local spatial distributions within the lake.

PHYLOGENETIC POSITION OF *A. AQUATICUS* (ISOPODA) FROM LAKE OHRID EVIDENCED BY MTDNA AND ALLOZYME DATA

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The Balkan Lake Ohrid, similarly to others from the set of "ancient lakes", preserves numerous endemic species and genera of various freshwater animals, but also is inhabited by widespread species which can however be locally differentiated on a morphological and genetic level. It appears that this diversity has most likely been caused by continuity of existence, exceptional geological age and relatively long isolation of the lake from outside water systems.

In addition to the three endemic isopod species, Lake Ohrid, is inhabited by *Asellus aquaticus* Linnaeus, 1758 which is widely spread throughout most of Europe.

Asellus aquaticus is found in freshwaters habitats such as slow-flowing rivers, streams and standing waters, particularly with muddy bottoms and abundant flora. It can survive even in brackish waters and is an excellent example of a plastic eurytopic species which can easily adapt to the new environment. The species is highly tolerant to organic pollution and has been used as an indicator of water quality.

Major parts of its range are inhabited by the type subspecies *A. aquaticus aquaticus*. However, the Balkan Peninsula is known by the extensive subspecific differentiation of this taxa. According to some authors, Lake Ohrid is inhabited by the morphologically distinct *arthrobranchialis* series, for which even a separate species *A. arthrobranchialis* was established.

This study aimed to show the position of Ohridian *A. aquaticus* species in comparison to Polish (mtDNA sequences and allozyme data) and other European populations (mtDNA sequence data from GenBank).

MOLECULAR-GENETIC STUDIES OF BAIKAL FISH EVOLUTION: SOME RESULTS AND HYPOTHESES

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Molecular-genetic studies of evolution of Baikal fishes have been carried out in the Limnological Institute of the Siberian Division of the Russian Academy of Sciences since the late 1980s. Two groups of Baikal fishes are studied more intensively – sculpins and whitefishes. The choice of these groups is stipulated by their peculiar characteristics: 1) These species dominant in ichthyomass are the only representatives of fishes in the open Baikal playing an important role in the lake ecology; 2) a high level of morphological diversity and adaptation to Baikal conditions is characteristic of these species, and 3) these and other peculiarities allow the use of sculpins and whitefishes as patterns for evolutionary studies.

Even the first results on molecular-genetic studies made a breakthrough in opinions on evolution and system of whitefishes. It appeared that the Baikal lacustrine whitefish is the closest relative of Baikal omul, and morphological similarity with omul representatives as the result of convergent evolution. The analysis of molecular data of whitefishes inhabiting Pribaikalye basins allows us to suppose that Lake Baikal is

situated in the center of the territory where two stages of fast cladogenesis took place during global glacials causing first the appearance of branches of the entire genus *Coregonus* and then its more diverse group of true whitefishes. An ancestor of the pair Baikal lacustrine whitefish/omul is likely to have inhabited Lake Baikal at least since the formation of the group of true whitefishes. Polymorphism analysis of their mtDNA, however, showed that both species and their numerous populations are very young.

Studies of phylogenetic relationships of Baikal sculpins were also effective. The most important results of phylogenetic analysis are estimates of evolutionary age and regularities of evolution of mitochondrial genes of these species. Results show that the age of the youngest ancestor of the sculpin complex spans 2-3 mln years. It is supposed that divergence of Baikal sculpins still continues intensively. The analysis of population structure of some species revealed a high level of intraspecific genetic subdivision of Baikal Cottoidei which also confirms the hypothesis that the process of speciation is ongoing.

Comparing regularities of evolution of studied groups of species, one should note that according to molecular-genetic data, time estimates to the nearest common ancestor are approximately equal for Baikal sculpins and whitefishes. Thus, for the period when species, genera and families were formed in sculpins, there were no even long-term existing populations of whitefishes. It is likely that the young age of the pair lacustrine fish/omul and their populations is attributed to peculiar features of ecology of whitefishes which caused repeated formation of pelagic and benthic forms similar to current species existing in Lake Baikal.

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COMPARATIVE POPULATION GENETICS OF STENOTOPIC ROCK-DWELLING LAKE TANGANYIKA CICHLID FISHES: SPECIES-SPECIFIC PATTERNS OF POPULATION STRUCTURE ACROSS A LARGE SANDY BAY AND ALONG CONTINUOUS ROCKY HABITAT

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Within a species, certain biological traits, such as ecological specialization, mobility, philopatric behavior, territoriality and mating system might influence the extent of population subdivision beyond the effect of external environmental influences and isolation by distance. In various organisms, comparative analyses of phylogeography and population structure have proven extremely powerful in the discrimination of external and species-specific factors in evolutionary processes. Due to their high eco-morphological and behavioral diversity, Lake Tanganyika's cichlid fishes provide an excellent model system for evaluating the relative importance of intrinsic and extrinsic forces shaping species-specific population structure and phylogeographic patterns. It has been suggested that drastic lake level fluctuations promoted allopatric diversification among populations by causing recurrent cycles of population fragmentation accompanying lake level rises and secondary contact during low water levels. However, comparative population genetics of several sympatric rock-specialized cichlid species in the very south of Lake Tanganyika reveals idiosyncratic patterns of population structure with differences in the responses to a large sandy bay, which acts as an important dispersal barrier in all rock-dwelling species studied

so far, and regarding dispersal along mostly continuous rocky habitat. In addition besides the lack of any common population genetic pattern among the rock-specialists, the population structure observed in some species contrasts with predictions based on the available information on behavior and ecological requirements. The findings of this comparative approach indicate that in stenotopic rock-dwelling cichlid species large-scale phylogeographic patterns are determined mainly by habitat structure and lake level fluctuations, whereas fine-scale population structure and demography are modulated by species-specific ecologies.

NUCLEAR MOLECULAR DIVERSITY AND MORPHOMETRY OF FRESHWATER CRABS FROM THE MALILI LAKES (SULAWESI, INDONESIA)

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The Malili lakes are located in the central highlands of the Indonesian island Sulawesi. These lakes are considered ancient limnic systems and therefore a very interesting case study for studying differentiation and speciation processes. First descriptions of the crab fauna from the Malili lakes took place in the beginning 20th century. The last taxonomic revision of freshwater crabs from Sulawesi revealed the existence of five gecarcinucid freshwater crab species in three genera within the three great lakes of the system, Matano, Mahalona and Towuti. One of these

species, the morphologically and ecologically well defined *Nautilothelphusa zimmeri* Balss, 1933 is the only species encountered in all three lakes. Mitochondrial genetic data from the 16S rRNA and the cytochrome oxidase I gene suggest that two of these lake populations (from lakes Mahalona and Towuti) are more closely related to the sympatric *Parathelphusa ferruginea* Chia & Ng, 2006 than to conspecifics from Lake Matano. The present study investigates relationships of *N. zimmeri* and *P. ferruginea* based on nuclear DNA by comparing amplified fragment length polymorphism (AFLP) and cloned sequences of the internal transcribed spacer 2 (ITS2). Furthermore, morphometric data are assembled to consolidate results from the molecular markers.

DISTRIBUTION OF *DREISSENA* LARVAE IN LAKES OHRID AND PRESPIA, MACEDONIA

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The presence, distribution and abundance of *Dreissena* larvae in the plankton of lakes Ohrid and Prespa were studied. Quantitative samples were collected in two seasons July and October from 6 sites in Lake Ohrid and 3 sites in Lake Prespa. The *Dreissena* larvae were found at different hauled lengths of the water column at all sites in both lakes. In

July the highest abundance was recorded at Trpejca (the southernmost part) reaching a mean value of about 2200 ind./m³ at length from 20 m to the surface. High abundances were recorded in the north-east part of the lake (Kosnosglobna and Pristaniste) but at higher lengths from 10 to 45 m. In October, the larvae abundance decreased. The highest abundance were recorded again in the south-east part of the lakes (Trpejca and Metropol, mean value of about 630 ind./m³) but at deeper parts: hauled lengths from 10 to 50 m. While in the north-east part (Sateska, Kosnosglobna and Pristaniste) the larvae were most abundant in the surface layer (up to 10 m). In July in Lake Prespa, very high abundances were recorded at Kazan: about 44700 ind./m³ at length 20-26 m, and mean value of about 21600 ind./m³ at length from 20 m to the surface. In October the abundances were considerably lower with highest values at the surface layer (8-0 m) reaching a maximum of 62 ind./m³ at Stenje. The results were compared with seasonal data from previous years and the reasons for the present distribution were discussed.

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POTENTIAL MECHANISM OF SURVIVAL OF THE BACTERIA OF THE GENUS *ENTEROCOCCUS* IN LAKE BAIKAL

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During the last few years the role of bacteria of *Enterococcus* has been attracting much attention not only due to their wide distribution in nature but due to the development of a resistance to many antibiotics, to drying, to light, to low temperatures and also to the resistance of physical, chemical and biological factors. Now the interest in these bacteria has increased because of their epidemiological importance. Enterococci are isolated from the clinical material under different pathological conditions. Most enterococci can enter the VBNC state under adverse environmental conditions. According the data nonculturable *Enterococcus faecium* cells are metabolically active and capable of resuming active growth. Oligotrophy is the main parameter inducing the VBNC state in these bacteria. Lake Baikal is a unique source of drinking water.

The main task of the research was to detect enterococcal contamination in the water Lake Baikal and to investigate the survival mechanism of the bacteria genus *Enterococcus* in the conditions of Lake Baikal. Morphological and molecular methods were used to detect the bacteria of the genus *Enterococcus* in the water of Lake Baikal. The mechanism of survival of enterococci was determined by experiments (cell number), by microscopy (ESEM and TEM).

The first expedition to Lake Baikal was in August 2005 in order to investigate the detection of enterococci contaminations. Microbiologically the bacteria of the genus *Enterococcus* were detected at the influents, especially at the surface and at medium depths. Bacteria of the genus *Enterococcus* were detected by molecular biological methods at influents and control points, especially further away at greater depths. During the expedition the enterococcal DNA was generally found at various sampling points without positive microbiological cultivation data. A viable but nonculturable state (VBNC) was hypothesized and experimentally verified under laboratory conditions.

Enterococcus faecium isolated from Lake Baikal were stressed by starvation, light and cold. Our experiments produced the following. The cells of enterococci formed nonculturable states. After addition of nutrient and incubation with increased temperature cells of enterococci could be resuscitated. ESEM and TEM microscopy indicated structural changes in cell shape (cell wall thickness) and size. Statistical evaluation by clustering variable data obtained by TEM microscopy helps us to understand the morphological variances. The cells of the bacteria of the genus *Enterococcus* are probably not eliminated in Lake Baikal and can survive adverse conditions and fall in VBNC state. Lake Baikal is likely to be a reservoir of dormant enterococci in lower layers or sediments of the lake and enhanced persistence in environment and in technological processes such as drinking water disinfection.

**EVOLUTIONARY HISTORY OF ANCIENT LAKE TITICACA: LESSONS
FROM THE *HELEOBIA* SPECIES FLOCK (GASTROPODA:
RISSEOIDEA)**

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Lake Titicaca arguably constitutes the only South American ancient lake with an estimated age of 2-3 My. The lake is situated in the tropical zone of the central Andes of Peru and Bolivia at an altitude of 3809 m. It is characterized by a bipartite basin with a total length of 178 km, a surface

are of approx. 8,500 km², a mean depth of 105 m and a maximum depth of 284 m.

Despite its considerable size, the biodiversity of Lake Titicaca is relatively low. There are approx. 500 species of animals and plants recorded from the lake to date with some 60 of them considered to be endemic. Several researchers have suggested that this relatively low degree of biodiversity might be due to the fact that the initial faunal components were of tropical origin and that only few species were able to adapt to high mountainous life. In addition, Pleistocene water level changes of up to 140 m may have had a great impact on the lake's biodiversity.

Nonetheless, some higher taxa such as molluscs, amphipods, ostracods, fishes, and sponges are characterized by a relatively high degree of endemic elements and some ancient lake species flocks were proposed. One of the largest of them is made up of 14 nominal species of the microgastropod genus *Heleobia* (family Cochliopidae).

Though *Heleobia* s.l. shows a strongly disjunct distribution (occurring in South America, northern Africa and Europe), our molecular data show that *Heleobia* s.s. is restricted to Lake Titicaca and its greater vicinity.

Molecular clock analyses indicate that the *Heleobia* species flock in Lake Titicaca is very young (i.e., only a few hundred thousand years old at maximum). Moreover, despite a fair degree of heterogeneity in the COI gene within *Heleobia* s.l., none of the nominal species can be separated genetically due to extensive paraphyly.

Interestingly, researchers recently found similar patterns in the killifish "*Orestias agassii* species complex": relative young age of most taxa and shared haplotypes among different nominal species.

The patterns observed in *Heleobia* do not support the hypothesis that the biodiversity of Lake Titicaca is limited by the climatic conditions of the lake and future studies will have to show whether the onset of

diversification within the *Heleobia* species flock in Lake Titicaca can be linked to the recovery from catastrophic late Pleistocene lake level changes.

**MICROSPORIDIAN PARASITES OF *GMELINOIDES FASCIATUS*
(STEBBING) IN LAKE BAIKAL, SIBERIA: POSSIBLE MECHANISM OF
PARASITE DRIVEN HOST SPECIATION**

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Here we report differential infections of amphipods *Gmelinoides fasciatus* with microsporidia in Lake Baikal and argue that this may be an important mechanism maintaining inter-population barriers. We have indeed found that different populations differ dramatically both by species composition of microsporidian parasites and by infection rate. This is the first such study in Lake Baikal. Lake Baikal is one of the most ancient lakes of our planet. Species diversity of organisms inhabiting the lake is very large. Such extremely high diversity arouses enormous scientific interest. In order to better understand this problem we used molecular genetic methods to investigate four distinct populations of *Gmelinoides fasciatus* characterized by negligibly small gene flow which could lead to their eventual speciation.

We examine the role of one possible candidate into this discriminating force, the microsporidians. They are unicellular obligate intracellular parasites of eukaryotes. They infect nearly all of the invertebrate phyla as well as all classes of vertebrates. It is important that some microsporidian species were shown to be able to modify the behavior and viability of their hosts. Some of them can significantly modify the hosts' ecological strategy. If microsporidia are causing a population to divide into separate populations it means that species composition of microsporidia and infection rate differ between them significantly. The easiest way to distinguish between different species of microsporidia is to use genetic analysis.

Therefore we undertook the analysis of the distribution of microsporidian species along the shore of Lake Baikal and compared it to the distribution of the hosts' genetic diversity. When investigating population structure one of the baikalian amphipod species *Gmelinoides fasciatus* we found that the distribution of them in Baikal Lake is subdivided into four geographically continuous regions inhabited by four distinct populations. Gene flow between the populations is impeded significantly in spite of the absence of geographic barriers between the populations. High degree of genetic differences between the adjacent populations cannot be explained with geographic barriers or other abiotic factors alone: there are no systematic long-term ecological differences between the areas occupied by distinctive populations, nor are there any important geographic discontinuities between them. Separate populations have their own demographic history. Therefore it is possible that in addition some biological disruptive influence played a role along with other factors. In this case such biological factors should differ between the populations at least as much as the populations differ from each other genetically.

We found that *Gmelinoides fasciatus* is infected by six microsporidia species. From our analysis it is absolutely certain that Northern-Central population is infected only by one species, whereas South-Western population is infected by all six species and South-Eastern by two species. This supports our assumption that microsporidia in Lake Baikal may be an important driving force of speciation. It is possible that microsporidia in Lake Baikal may have separated other species in the geologic past, for example molluscs. Further research is needed in order to prove or disprove this hypothesis.

Our main conclusion is that there is an intriguing possibility of a parasite stimulating differential stability in *Gmelinoides fasciatus* host and thus maintaining the boundary between populations of the latter and may be even causing speciation in Lake Baikal.

ENDEMISM AND COSMOPOLITISM IN DIATOMS FROM THE LARGE LAKES: A CASE STUDY OF *AMPHORA* (BACILLARIOPHYCEAE)

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Large ancient lakes are known as centers of speciation and endemism. Endemism in ancient lakes is present in all groups of organisms. Diatoms are one of the best studied organisms in ancient lakes, showing a

relatively high degree of endemism. In this study published data were compared with data obtained from observations of the *Amphora* species (Bacillariophyceae). According to compiled published data for the lakes Ohrid, Baikal, Tanganyika, Hövsgöl and Laurentian Great Lakes, a total of 62 *Amphora* taxa has been recorded. From these, 36 taxa are considered widespread and recorded outside the lakes of interest, while only 19 taxa are considered as potential endemics. The highest number of endemic taxa is recognized in Lake Baikal (11 taxa), while lakes Tanganyika and Michigan have three endemic *Amphora* species each. No endemic species of *Amphora* have been recorded for Lake Hövsgöl, while in Lake Ohrid, only two species were considered endemic.

Recent observations, however, indicate that there are at least 100 *Amphora* species in these lakes. A total of 54 taxa is considered endemic (known only from one lake), while one species, *Amphora pediculus* sensu lato, has been observed in all of the investigated lakes. However, the population from Lake Tanganyika has slightly different areola ultrastructure and its allocation to *A. pediculus* is questionable. *A. pediculus* sensu Lake Tanganyika is characterized by small-sized valves (length below 10 µm; width below 3 µm) and is hard to differentiate in LM. The highest number of endemic species of *Amphora* has been recorded in Lake Baikal (20 taxa or 60.6%), although the highest percentage of endemic species has been recorded from Lake Tanganyika (84.2 %).

In each investigated lake, a characteristic species flock has been observed. In Lake Baikal there is a species flock centered around *A. ovalis/mongolica*; in Lake Tanganyika a species flock centered around *A. turbida*; in Lake Ohrid a species flock centered around *A. copulata*; and in Lakes Hövsgöl and Michigan a species flock centered around *A. affinis*. Such a distribution of taxa indicates intralacustrine speciation within the genus *Amphora* from ancient lakes.

APPLYING FISH AND CARD-FISH PROTOCOLS TO STUDY SEDIMENTS OF LAKE BAIKAL

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We present the first data obtained from our experiments which aimed to apply FISH (fluorescence *in situ* hybridization) protocol to study sediments collected in the south part of Lake Baikal in July, 2005. To count hybridized cells and total amount of microbial cells (TAMC), dyed by fluorescent dye DAPI (4',6-diamidino-2-phenilindol), a special program Image Test was applied. The collected core of sediment was 20 cm long and several dot horizons were studied. Average amount of TAMC was $4,4 \cdot 10^9$ cells per 1g (cl/g) of dried sediment, max – $1,74 \cdot 10^{10}$ cl/g (10-11 cm) and min – $2,83 \cdot 10^{10}$ cl/g (1-2 cm). We tested different temperatures (35-43°C) and different time of hybridization (2-4 hours). Best results were obtained at 35 °C and 2 hours of hybridization. Following microorganisms was detected in the sediment:

- Archae: average amount is $2,1 \cdot 10^8$ cl/g, max. – $8,2 \cdot 10^8$ cl/g (10-11 cm), min. – $1,3 \cdot 10^7$ cl/g (4-5 cm, 15-16 cm).
- Eubacteria: average amount is $2,6 \cdot 10^8$ cl/g, max. – $1,3 \cdot 10^9$ cl/g (10-11 cm), min. – $7,3 \cdot 10^5$ cl/g (0-1 cm).
- Plankomycetes: average amount is $5,2 \cdot 10^8$ cl/g, max. – $1,1 \cdot 10^9$ cl/g (10-11 cm), min – $1,1 \cdot 10^7$ cl/g (2-3 cm).

- Sulphate-reducing bacteria (average amount is – $3,1 \cdot 10^8$ cl/g, max. – $6,1 \cdot 10^8$ cl/g (4-5 cm), min. – $3,0 \cdot 10^7$ cl/g (2-4 cm) and there was no sulphate-reducing bacteria at 10-11 cm.

From this data we can see, that the max. amounts of TAMC, Archae, Eubacteria and Plaktomycetes correspond to 10-11 cm of the sediment. From the other hand, sulphate-reducing bacteria was not detected at this level and was plentiful at 4-5 cm, having a low portion of its population at 10-11 cm.

We also tried to apply FISH and CARD-FISH protocols to study sediments, collected in the area of Bolshoe Goloustnoe village in March, 2008. A 65 cm core was analyzed. Max. numbers of TAMC occurred in the first 5 cm, the highest ratio was $2,8 \cdot 10^{10}$ cl/g at 0-5 cm and then rapidly dropped to min. $1,3 \cdot 10^9$ cl/g at 35-40 cm with a comparatively insufficient increase at 50-55 cm and drop at 60-65 cm ($6,6 \cdot 10^9$ cl/g, $4,4 \cdot 10^9$ cl/g correspondingly). Data obtained from the experiments allowed us to see Archae at 60-65 cm ($3,4 \cdot 10^3$ cl/g) and sulphate-reducing bacteria at 15-20 cm ($1,4 \cdot 10^4$ cl/g), 50-55 cm ($3,9 \cdot 10^3$ cl/g). This is surprising, taking into account that the area of Bolshoe Goloustnie village has strong organic flows in the water and sediment.

We conclude that it is possible to work with sediments collected in different parts of Lake Baikal and to get good results, using FISH and CARD-FISH protocols. However, its specificity must be taken into account.

COMPOSITION AND DYNAMICS OF MICROBIAL COMMUNITY IN LAKE OHRID

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The ancient Lake Ohrid (the time of the formation of its basin and the lake itself is near the tertiary epoch of the Planet's geological history) is rich with endemic forms due to the continued existence of the lake, to the constancy of the complex of living circumstances in it and its geographic isolation. With all those characteristics, the Lake Ohrid is the only one in Europe, rare in the world as a natural phenomenon and it is considered natural monument, ecological and cultural treasure with worldwide importance.

The microorganisms in the water environment have the chief role in the process of transformation of the organic material, and in the functioning of the ecosystem as a whole. Knowing the composition and the dynamics of their population is a realistic indicator for determination and forecast of the condition in the aquatic ecosystem. The bacteria are very important indicators for a determination of the level of cleanliness of the analyzed water. As first pointers for eutrophication, they have primary importance within the frames of the hydrobiological research.

The composition and dynamics of the microorganisms in the Lake Ohrid have been monitored for longer period of time (1996-2006). The research included parameters of ecological (the presence and quantity of more important physiological groups of bacteria which indicate on the specificity of the organic pollutants) and sanitary aspects of the conditions of the

water (coliform bacteria, fecal indicators) for determination of its hygiene status.

The retained results from the research revealed that the presence and the composition of the microorganisms is space and season variable, depending on the abiotic and biotic factors, the level of eutrophication and anthropogenic influence. Generally, the lake is in the category of clean waters with a domination of oligotrophic bacteria. The relatively low and unimportant quantity of all analyzed groups of bacteria in the pelagic zone and the majority of the localities in the littoral, indicate that the process of transformation of the organic material is rather slow.

The peladic zone is still oligotrophic balanced as a result of the ability for self-purification; it is still not under the influence of communal, industrial and waste waters of the cost rich of pollutants and nutrients which can result in rapid eutrophication of some parts (low-level) of the littoral zone, such as the areas before the river's delta and settlements.

This condition entails the necessity of long-run control of the quality of the water and prevention of the aquaterium of the Lake Ohrid.

BACTERIA AS AN INDEX OF WATER POLLUTION OF THE LAKE PRESPA

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The Lake Prespa, under some natural and anthropogenic factors, during the last decades manifests changes in the morphometrics of the basin

and quicker process of eutrophication. The rapid decrease in the level of the lake, due to the intense usage of the water, resulted in decrease in its volume and in deterioration of the water's quality – which affects on the changes of the entire ecosystem. The tributaries are recipients of industrial and communal waste waters.

The changes of the space and time distribution of the total number of heterotrophic bacteria and of some physiological groups of bacteria (proteolytic, amilolytic, lipolytic, phospho-mineralizing, phospho-mobilizing, nitrogen fixing and cellulolytic bacteria), as well as of the total number of coliform bacteria and the presence of *Escherichia coli* were monitored in the period 2008-2009 in the waters of the Lake Prespa and its tributaries.

There have been determined important differences in the quality of the waters from the analyzed localities, as well as in regard to the composition of the communities. In the rivers there have been registered rather higher values of all analyzed groups of bacteria, with maximums in the River Golema (Big). During the summer period it has been observed that the riverbed of the locality at Carev Dvor is dry. The samples were taken in the town of Resen and it has been indicated that in that moment in the river waste communal and industrial water was present. The results from the bacterial researches indicate the same, with substantial mass presence of coliform bacteria and *E. coli*. The relatively high values of the other bacteria groups in the waters of the river and in the coastal zone of the lake near Asamati can be explained with the large quantities of deposited apples which the producers were unable to sell.

The large surfaces of the coastline where the water is retrieving are entirely covered with macrophytes (entire meadows) which definitely contribute to the increased bacterial presence, especially increasing the

presence of the cellulolytic bacteria in all analyzed localities. Substantial organic encumbrance is registered in the pelagic zone of the Lake. For prevention of the ongoing process of eutrophication of the Lake Prespa and to enable its revitalization certain measures have to be undertaken, such as increased control of the waters that flow into the lake, to be cautious of the biological minimum of the water in the rivers, which also during the summer period are used for irrigation without any control. The present pollutants should be eliminated, or at least decreased and a proper regime for prevention from allochthonous pollutants should be implemented. Finally, a constant monitoring of the quality of the water should be conducted.

**SCIENTIFIC COLLABORATION ON PAST SPECIATION CONDITIONS
IN OHRID (SCOPSCO): RECENT AND FOSSIL OSTRACODES FROM
LAKE OHRID AS INDICATORS OF PAST ENVIRONMENTS: A
COUPLED ECOLOGICAL AND MOLECULAR GENETIC APPROACH
WITH DEEP-TIME PERSPECTIVE**

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The group of Ostracoda is an ideal model group to study speciation. Extant species can be examined as well as fossil calcified valves in the lake sediment. In the second deepest lake of Europe, Lake Ohrid (289 m), 52 ostracode species are described in literature of which 33 are believed to be endemic to the lake itself. The maximum of ostracode species diversity is found at around 40 m water depth.

On the basis of taxonomy, autecology and molecular genetic methods on recent ostracodes, and using studies on fossil assemblages, we intend to make a contribution to the bundle project “Scientific Collaboration On Past Speciation Conditions in Ohrid (SCOPSCO)”. Overall goals are to (1) clarify the origin and the age of the ancient Lake Ohrid, and to (2) investigate the reasons of speciation events.

We present preliminary data from our first field work campaign in spring 2009. We sampled three depth transects to a water depth of 282 m with a multi gravity-corer (3x 78.5 cm²). At the sampling site we measured the physico-chemical parameters of the surface and bottom lake water and the properties of the sediment to describe the ecological niche of the identified species. First results show that we still encounter a diverse fauna in water deeper than 130 m (e.g., *Candona hadzistei*, *Cyprina obliqua* and *Leptocythere* sp. can be found).

Samples in the shallow littoral and in aquatic habitats of the catchment of the lake (springs, flooded fields and agricultural ditches) will be also included in a metadatabase to interpret fossil assemblages in longer sediment records.

**MORPHOLOGICAL PATTERNS AMONG ALLOPATRIC AND
SYMPATRIC POPULATIONS IN THE LAKE TANGANYIKA CICHLID
GENUS *TROPHEUS***

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Lake Tanganyika contains the oldest and most complex flock of cichlid fishes. They are excellent subjects for the study of explosive speciation and adaptive radiation. Many species are subdivided into arrays of geographic morphs, each colonizing particular sections of the shore line. Their present distribution was shaped by a series of lake level fluctuations. One of the best examples of this phenomenon is the genus *Tropheus*, counting about 120 distinctly colored populations and sister species, some living in sympatry. There is strong evidence from ecological and genetic studies that *Tropheus* is not capable of covering large distances across unsuitable habitats or open water, as a consequence of its pronounced habitat specificity, site fidelity, and territorial behavior.

Our study analyzes phenotypic variation in three *Tropheus moorii* populations living in sympatry with its sister species *Tropheus polli* compared to three allopatric *Tropheus moorii* populations. We use geometric morphometrics based on Procrustes superimposition of 19 morphological landmarks to quantify overall bodyshape. Canonical variate analysis and principal component analysis are used for data description. In addition to comparisons of overall shape we apply discrete measurements in form of interlandmark-distances (ILD).

Although overall morphology appeared similar, we found significant shape differences and patterns of morphological variation in allopatric populations in comparison to sympatric populations. All three sympatric *Tropheus moorii* populations showed larger fins but smaller heads and smaller eyes compared to the allopatric populations. Results showed consistent patterns of morphological distinctness of all *Tropheus moorii* populations which co-occur with *Tropheus polli* compared to *Tropheus moorii* populations living without a potential competitor.

LONG-LIVED LAKES AS LABORATORIES OF MOLLUSK EVOLUTION: A CASE STUDY FROM THE MIOCENE DINARIDE LAKE SYSTEM

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Since the 19th century the Southeastern European Neogene long-lived lakes such as Lake Pannon or Lake Slavonia (“Paludina” lake) have been very well known for their manifold endemic fauna, providing reportedly Charles Darwin with the first hand evidence to support his evolutionary theory. The present investigation takes place in the adjoining Miocene Dinaride Lake System, an long-lived, endemic lacustrine environment of about 75,000 km² maximal extent, positioned on the huge, NW-SE striking land mass, a paleo(bio)geographic barrier between the Central Paratethys and the early Mediterranean Sea. The often very thick lacustrine sediments filling the basins provide an exceptional record

associated with a manifold depositional environment (coals, volcanic ashes, siliciclastics, tuffaceous carbonates). The time resolution based on a modern, integrated stratigraphic approach now provides a unique opportunity for critical proof of the evolutionary parameters observed in the basins.

The Dinaride Lake System mollusks are nearly completely endemic, originating apparently from autochthonous speciation and radiation events. In total, about 190 species level taxa, grouped into 36 genera, became known from there. Among these, the most diverse genera are prososthenid (*Prososthenia*) and melanopsid (*Melanopsis*) gastropods, followed by dreissenid bivalves. Consequently the most eye-catching radiations of mollusks are represented by *Melanopsis* and *Prososthenia*. These radiations and the represented morphologies probably have striking counterparts in the younger faunas of Lake Pannon. Similar convergences have been documented within dreissenid bivalves which developed unrelated morpho-pairs in the Dinarid Lake System and Lake Pannon. Whereas some genera such as *Orygoceras* are endemics to both lake systems, the large-sized, limpet-like gastropods such as lymnaeid *Delminiella* or presumable ancylid *Clivunella* are restricted exclusively to the Dinaride Lake System.

The study represents a contribution to the Austrian FWF Project P18519-B17: "Mollusk evolution of the Miocene Dinaride Lake System".

EFFECTS OF CLIMATE CHANGE ON POPULATION STRUCTURE AND GENETIC DIVERSITY OF LAKE TANGANYIKA CICHLIDS

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Little is known about differential effects of climate change on various animal taxa. The MOLARCH project explores the influence of climatic history on patterns of genetic differentiation in endemic species flock radiations of two ancient lakes (Lake Baikal and Lake Tanganyika). Our research group studies the effects of climatic changes on Lake Tanganyika cichlids. Population structure and speciation patterns of lacustrine cichlids are known to be greatly affected by lake level fluctuations which also influence habitat stability. We sampled four different cichlid species in stable habitats along steep shores where population structure should not have been affected even by large lake level fluctuations in the past. Samples were also taken in instable habitats along shallow shores where repeated admixture and separation of neighboring populations should have left visible traces on population structure. Using two mitochondrial genes we found significant differences in population structure, patterns of genetic diversity and population demography among the studied species.

**NICHE OR NEUTRAL? SCALE-DEPENDENT PROCESSES OF
COMMUNITY ASSEMBLY IN ENDEMIC GASTROPODS OF LAKE
TANGANYIKA, EAST AFRICA**

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Recent theoretical models have rekindled interest in whether communities of species are assembled by niche-based sorting or ecologically neutral factors such as dispersal limitation. We have tested the roles of both dispersal potential and environmental factors in structuring shoreline assemblages of snails in Lake Tanganyika at multiple spatial scales. We quantified assemblages at 22 sites, and evaluated whether assemblage similarity was a function of environmental differences or geographic distance at multiple spatial scales. Within sites (5-25 m), snail assemblages shift considerably along depth gradients but not across comparable lateral distances, indicating strong responses to environmental characteristics. At larger scales (0.5-27 km), environmental similarity and distance between sites consistently predicts assemblage

similarity across sites. Our results indicate that the factors governing assemblage structure are strongly scale-dependent; niche-based mechanisms act across many spatial scales, and ecologically-neutral processes become important only at large spatial scales.

MOLARCH MOLLUSCS AND THE *LAVIGERIA* LEGACY: HOW ACCURATELY DO GASTROPOD POPULATION GENETICS REFLECT PALEOGEOGRAPHY OF TANGANYIKA'S SHORELINE?

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The MolArch project aims to use comparative cross-taxonomic data from endemic species in ancient lakes to explore genetic responses to climate changes and test for common physical drivers of diversification. Molluscs are ideal for this kind of study as their ecology ties them closely to specific benthic habitats, potentially making them faithful reflections of past environments, and their evolution is more likely tied to physical drivers than exceptional mechanisms such as sexual selection, known in many endemic fishes.

Lavigeria gastropods are the most common invertebrate grazers in the rocky benthos of Lake Tanganyika, forming a species flock of approximately 45 species with sympatry of up to 6 species at a site. Here we present results of population genetic studies of several of the ovoviviparous *Lavigeria* species that contrast in life history strategy. Populations of *L. grandis* and *L. nassa* were collaboratively sampled along the central Tanzanian shoreline as part of the MolArch project, which included sampling of fish, ostracods and shrimp from the same sites. Although *L. grandis* and *L. nassa* are sister species found in sympatry throughout the lake, they have contrasting reproductive strategies which are likely to influence levels of gene flow among populations. Haplotype networks and population genetic parameters of COI DNA sequence indicate that *L. grandis* have less population genetic structuring, despite a more K-shifted life history strategy, whereas *L. nassa* have far greater population structuring and haplotype diversity, despite a more r-shifted reproductive strategy. As a palaeogeographic tracer, the signal from *L. nassa* is likely to be more sensitive. Comparison of genetic patterns among these snails and the other taxa studied at these sites will test this prediction.

USE OF BIO-LOGGING SYSTEMS TO UNDERSTAND DIVING BEHAVIOR OF BAIKAL SEALS, LAKE BAIKAL

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Bio-logging Science is an important system science for understanding swimming behavior of aquatic animals and their environmental condition. We have developed the advanced data loggers and camera logger, and used them for various kinds of animals such as whales, seals, sea turtles, sea birds and fishes. Diving behavior of six Baikal seals (*Phoca sibirica*) has been studied using the advanced data logger (UWE1000-PD2GT: 22 mm in diameter, 124 mm in length, 92 g in the air; Little Leonardo), the advanced digital still logger (DSL-380DTV: 22 mm in diameter, 138 mm in length, 73 g in the air; Little Leonardo) and the original automatic releasing system with timer and VHF radio signals. The seals dived almost continuously, to an average of 68.9 m, with dives deeper than this (>150 m) concentrated around dusk and dawn. They showed distinctly different diving patterns between day and night in terms of swimming speed profile and the sequential pattern in maximum dive depth. In the

daytime, dives were characterized by higher swimming speeds (mean 1.2 m s⁻¹) and upward-directed acceleration events. At night, dives were shallower around midnight and characterized by lower speeds (mean 0.9 m s⁻¹) and unidirectional deceleration events. We suggest that these differences reflect predation on pelagic fishes by seals using visual cues during the day and predation on swarming crustaceans by seals using tactile cues at night. The seals also showed different stroke patterns among individuals. To investigate buoyancy control systems on air-breathing divers, we conducted the experimental study of Baikal seals equipped with lead weights using an automatic releasing system. This study indicated that the seal increased its stroke rate in descent by shifting swimming mode from prolonged glides to more stroke-and-glide swimming, and decreased its stroke rate in ascent by shifting from continuous stroking to stroke-and-glide swimming. We conclude that seals adopt different stroke patterns according to their individual buoyancies. A simple physical model allowed us to estimate the body density of the seal from the speed and pitch (1027–1046·kg/m⁻³, roughly corresponding to 32–41% lipid content, for the weighted condition; 1014–1022·kg·m⁻³, 43–47% lipid content, for the unweighted condition).

DEMOGRAPHIC HISTORIES OF SELECTED CICHLID SPECIES FROM LAKE TANGANYIKA

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In the framework of the MOLARCH project, population samples (20 to 50 individuals) of 4 cichlid species (*Eretmodus cyanostictus*, *Tropheus moorii*, *Variabilichromis moorii* and *Perissodus microlepis*) were collected from 8 to 11 localities in Lake Tanganyika. Each locality was classified as either stable or unstable depending on the inclination of the shoreline: steep shorelines taken to be stable, gently sloping shorelines taken as unstable. This classification is due to the expected different impacts of the well-known water level fluctuations in Lake Tanganyika in these different shoreline profiles. In very steep areas, water level fluctuations are expected to result in dislocation of fish populations following the retreat in water level, but the amount of available habitat should remain approximately the same. Conversely, in very gently sloping areas, water level regressions will result in large areas becoming dry, and we thus expect fish populations from these areas to show traces of more variable demographic histories. We sequenced mtDNA genes (control region and CO1) and used MCMC sampling to infer the demographic histories of the different populations, and interpret the different outcomes in view of our expectations and the ecological and life history characteristics of the different species.

**ARE THERE ANCIENT LAKES ON THE TIBETAN PLATEAU?
PHYLOGEOGRAPHY OF *RADIX* SPP. (GASTROPODA,
LYMNAEIDAE) IN TIBET**

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Located in central Asia, the Tibetan Plateau is the largest and highest plateau in the world. On the plateau, there are about 1,600 lakes with an area greater than 1 km². The evolutionary and limnological history of these lakes is closely related to both geodynamics and to glaciations and climate. In contrast to the view that all extant lakes on the plateau have developed after the Last Glacial Maximum (approx. 18,000 B.P.), some more recent studies could demonstrate that certain lakes were continuously filled by sediments during the late Pleistocene and hence sedimentation was not interrupted during the Last Glacial Maximum. However, the information about lake age and history is still very poor and lakes rough age estimates are only available for very few lakes.

The aquatic gastropod genus *Radix* (Hygrophila, Lymnaeidae) is widely distributed on the plateau, and *Radix* spp. belong to the first invaders of a newly developed lake (e.g. after retreat of a glacier). In this study, phylogeographical patterns of *Radix* spp. on the Tibetan Plateau are investigated in order to give insight into Tibetan freshwater biogeography and the history of the Tibetan Plateau lakes.

Therefore, specimens of *Radix* from several lake systems were collected on an expedition to the eastern Tibetan Plateau in autumn 2008. These samples were investigated genetically by sequencing two mitochondrial genes (COI, LSU rDNA).

The results show an overall high genetic diversity of *Radix* on the plateau with remarkable differences between the central and eastern lakes. Preliminary molecular clock analyses suggest a recent colonization of the central plateau lakes by *Radix* spp., whereas the onset of diversification of *Radix* taxa in the eastern lakes may have started during the Pleistocene or even during the Pliocene. This data thus may warrant further investigations into the existence of ancient lakes on the eastern Tibetan Plateau.

PHYTOPLANKTON COMPOSITION IN TWO ANCIENT LAKES WITH DIFFERENT TROPHIC STATE

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Lake Ohrid and Lake Prespa, two of the few ancient, long-lived lakes of the world are situated in southeastern Europe, between Macedonia, Albania and Greece. Lake Prespa has no surface outflow and it is connected with Lake Ohrid by underground hydraulic connections.

Lake Ohrid is oligotrophic and Lake Prespa is in process of eutrophication and in the investigated period was in mesotrophic state.

Water samples were collected during 2001-2003 from the pelagic zone of Lake Ohrid on nine depths (0, 10, 20, 30, 40, 50, 75, 100, 150 m) and

from the pelagic zone of Lake Prespa on four depths (0, 5, 10, 15 m) by Niskin bottles, seasonally and with a monthly frequency of sampling in the summer period.

The investigations indicated large difference in the phytoplankton composition of these two lakes. In Lake Prespa, *Cyanophyta* and *Bacillariophyta* had the main role in the phytoplankton, contributing on average approximately 95% of the phytoplankton biomass, with very low percentage-wise presence of the other groups of algae. This presence of the taxonomic groups of algae is characteristic of lakes which are in the process of eutrophication. Unlike Lake Prespa, in Lake Ohrid *Chrysophyta*, *Chlorophyta* and *Pyrrophyta* had substantial contribution in the phytoplankton which is characteristic of the oligotrophic lakes while *Cyanophyta* and *Bacillariophyta* accounted for about 65% of the phytoplankton biomass.

The average percentage presence of the existent groups of algae in Lake Ohrid during the three research years indicated trend of a decrease of the participation of *Cyanophyta* from 29.48%, 26.84% and 11.56% in 2001, 2002 and 2003, respectively. On the other side, the percent of *Bacillariophyta* indicated trend of increase from 35.03%, 43.62% and 50.41% in 2001, 2002 and 2003, correspondingly. The same tendency of increase showed *Chrysophyta* from 6.47% in 2001, 15.27% in 2002 and 19.02% in 2003.

During the entire investigated period, in Lake Prespa a tendency of increased percentage presence of blue-green algae had been observed from 30,89% in 2001 and 31,71% and 55,6% in 2002 and 2003, in that order. Diatoms were presented with 63.39% in 2001, 65.41% in 2002 and 40.17% in 2003. The other groups of algae contributed unsubstantial part of the total density: *Chlorophyta* with percentage presence of 1.4%, *Chrysophyta* with 1.13%, while *Pyrrophyta* with 1.75% from the total

density – which is rather supportive argument of the notion that during the last few years in Lake Prespa there are occurring certain negative processes which result in aggravation of its trophic state.

The data regarding the phytoplankton community composition of Lake Prespa dated some 50 years ago are more consistent with the present schedule of the percentage presence of the existent groups of algae in the phytoplankton of Lake Ohrid, then to the present image of Lake Prespa.

Considerable difference in the phytoplankton community composition of the two lakes was evidenced during the summer period. In Lake Ohrid, *Cyanophyta* contributed 10% in 2001, 29% in 2002 and it was not present at all in 2003. On the other hand, in Lake Prespa in 2001 it amounted with 67%, in 2002 with 48% and in 2003 with 51%. *Bacillariophyta* in Lake Ohrid was present with 4% in 2001, 1% in 2002 and 6% in 2003 while in Lake Prespa its presence was quite higher, i.e. 28%, 51% and 35% in 2001, 2002 and 2003, in that order. As for the *Chrysophyta* it had great contribution to the phytoplankton community of Lake Ohrid (23%, 27% and 47% in 2003, 2001 and 2002, respectively), while in Lake Prespa it was evidenced with 1% in 2001 only, and the following years it has not been confirmed at all. *Pyrrophyta* had high presence in Lake Ohrid, i.e. 16% in 2002, and 49% in 2001 and 55% in 2003 while in Lake Prespa it had substantial contribution to the phytoplankton community in 2003 with 14% and in 2001 and 2002 it contributed with 3% and 1%, correspondingly.

All comparisons of the phytoplankton composition of the two ancient lakes noted above clearly indicate the changes of the percentage presence of different taxonomic groups of algae which occur during the process of eutrophication, hence the phytoplankton composition presents clear image of the lake trophic state.

THE HISTORY OF CHANGING ECOLOGICAL NICHES DURING SPECIATION IN ENDEMIC MOLLUSKS OF GENUS *BAICALIA*

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The genus *Baicalia* (Martens, 1876) includes four species with subspecies and belongs to the endemic family Baicaliidae of Lake Baikal. All species of this genus have different types of breeding behavior: attaching eggs to sandstones (*B. dybowskiana*, *B. carinata rugosa*), attaching them onto hard surface of rocks or stones (*Baicalia turiformis*), attaching eggs to sand particles (*B. carinatocostata*) and finally the peculiar behavior of *B. carinata*, attaching eggs to the shells of snails of the same species. Presence of numerous biological and ecological investigations of these mollusks, their wide spread and low rate of migration along perimeter of littoral Lake Baikal provide a unique possibility to study scenarios of speciation.

Here we describe the studies of evolutionary history of baikalian mollusks using information about nucleotide sequences of mitochondrial cytochrome c oxidase subunit 1 gene fragment (mtCO1) and nuclear internal transcribed spacer of (ITS1) taking into account geographic factors.

Here we used haplotype minimum spanning networks and phylogenies to elucidate the history of spreading and scenarios of mollusks speciation within genus *Baicalia*.

Phylogenetic analysis successfully resolves two populations of *B. carinata*. Posterior probabilities of all major branches as estimated with

the program mrBayes are high enough to believe that this tree topology is well supported. As with ITS1, all species of *Baicalia* are significantly monophyletic. But the branching order of the mitochondrial tree is dramatically different if compared to the ITS1 phylogeny. *B. dybowskiana* and *B. turiformis* on the “mitochondrial tree” as well as on the mitochondrial minimum spanning tree are young and only depart slightly from the clusters of the two populations of *B. carinata*.

We used IM model in order to estimate demographic parameters, coefficients of migration and time since clades isolation. In the case of *Baicalia* it is safe to use this approach only for the pairs of species which appear as siblings both on the CO1 and ITS trees. In 3 cases the values estimated from ITS1 sequences are several times higher than the ones estimated by CO1. This corresponds to the discrepancies between the phylogenies and median networks when according to mitochondrial sequences the species appear inside one of the *B. carinata* haplotype clusters. This suggests that after the reproductive barrier between the species had been established, there was secondary contact marked by complete flush of one of the species' mitochondrial genomes with the genome of the other species. The comparison of curves shows that this was not simultaneous for different pairs of species but that the sand dweller *B. carinata* was the source of the sweeper genome. Using molecular clock hypothesis it is possible to date the mitochondrial transgression approximately to 0.5 MYA when rapid tectonic changes finally shaped the current ultra-deep Lake Baikal and one could expect rapid reshuffling of substrate types along the shore between the rocky steep ones and gentler sandy beaches giving more advantage to the sand dwellers.

The comparison of evolutionary history of taxa comprising genus *Baicalia* based on nucleotide sequences of two molecular markers: nuclear ITS1

and mitochondrial COI shows dramatic discrepancy in position between two species: *B. turriiformis* and *B. dybowskiana*. According to a phylogeny inferred from mitochondrial sequences in both cases, the taxa in question appear to be young branches or in-groups to the Eastern population of *B. carinata*. However on the tree inferred from ITS1 sequences both species are basal to the split between the *B. carinata* populations. This pattern of tree topology distortion is typical for a mitochondrial transgression when due to inter-specific hybridization a more numerous sister species comes into secondary contact with its rarer sibling. This may happen so that the mitochondrial introgression occurs but the nuclear introgression does not.

The hypothesis explaining discrepancy between mitochondrial and nuclear phylogenies in case of *Baicalia* would be that *B. dybowskiana* and *B. turriiformis* came into secondary contact with the *B. carinata* western population after the latter separated from *B. carinatocostata* and the eastern population became a separate entity. This could be due to a selective sweep caused by an environmental change as was experimentally proved for two *Drosophila* species by Niki et al. (1989) and/or due to one or more severe bottlenecks. Eastern ecological equivalent of *B. dybowskiana* *B. carinata rugosa* occurs fully allopatrically and is the youngest taxon of the genus according to both genes. One may speculate that as the species *B. dybowskiana* occurred on the West Coast much before the split between *B. carinatocostata* and *B. carinata*, and the second speciation could have happened very recently when this niche became available on the East Coast. This could not be echoed on the West Coast where the niche was already occupied by *B. dybowskiana*.

Since *B. carinatocostata* appears to be the most basal branch of the genus tree, one may conclude that the founder of the genus was a

successful sand-dweller breeding on sand. Indeed, its split from other Baicaliidae dates back approximately to 1MYA or more if we assume the Brown *et al.* (1979) estimate for average rate of molecular evolution in mitochondrial DNA as 2% per million years and remember that tMRCA is obviously smaller especially in cases of complicated demographic history of a population. The next extant species to appear was *B. dybowskiana*. Unlike its ancestor, this species has to travel to a rock to lay eggs, which returns it to the pattern common for the majority of the representatives of the family and thus is likely to be the plesiomorphy. Although regarded as sand-dweller, this species tends to occur on silted sand patches between boulders and does not have to travel far to find a suitable breeding ground. It is most abundant at Maloe More Straight where it becomes sympatric to *B. carinata* but never is found in the same samples with *B. carinatocostata*.

The next branch is rock dwelling *B. turiformis*. It lays eggs onto rocks and has peculiar type of feeding: it hangs on a sticky proteinacious thread to which the food particles attach. It forms extremely dense populations along the rocky shores mostly on the West Coast of the lake. The last major extant descendant from the common ancestor of *Baicalia* is *B. carinata*. This is the most numerous species occurring along whole perimeter of the lake, although on the West Coast its population is fragmented strongly by stony patches of bottom. *B. carinata* is split into two populations. Although the Northern boundary between the populations naturally coincides with Upper Angara River delta, the Southern boundary cannot be easily explained by geographical barriers alone. The eastern population is much more numerous due to the much higher availability of sandy bottoms. However, tMRCA estimates as well as minimal spanning tree suggest that the Western population is the ancestor, the east coast was colonized later by the migrants. The peculiar

breeding strategy of laying eggs onto shells of other snails may be considered to be the alternative derivation of the primitive trait of laying eggs on a firm surface. There are obvious advantages to very dense populations where this type of solid substrate for eggs is always nearby. Therefore this strategy relies on an increased flow of nutrients from the shore possibly due to increased erosion.

The youngest of all taxa is *B. carinata rugosa* inhabiting East Coast only. It is sand dweller traveling to stones to lay its eggs and thus it occupies the same or almost the same niche as *B. dybowskiana*. Since its taxonomic position is not yet finally agreed (Sitnikova), our data may be considered to be an argument towards the specific status of this taxon.

As the discrepancy between phylogenetic schemes based on mitochondrial and nuclear markers, there were at least two cases of historical mitochondrial transgression of the genus *Baicalia*. In both cases mitochondrial genomes of two relatively rare species: *B. turiformis* and *B. dybowskiana* were swept by mitochondrial genomes of *B. carinata*, the most numerous species, which may be found on rocks and boulders as well as on sandy gentle slopes most comfortable to them.

Similar processes were observed in other species but better studied in experiments on fruit flies, where it was shown experimentally that the transgression was more likely to happen if rare and less adapted (under current circumstances) species crossbreeds for short time with more numerous species while the “sweeper” genome offers some significantly advantageous traits.

LAKE SKADAR – BIODIVERSITY AND ENDEMISM OF A YOUNG ANCIENT LAKE

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A survey of the fauna and flora of Lake Skadar confirms that the lake harbours a rich biodiversity. Despite the fact that some biotic groups are poorly studied or not studied at all, approximately 1,670 native species are known from the lake, including 589 animals, and at least 36 species are endemic, including 34 animals.

Though an extensive bibliography on the lake had been recently compiled, so far no attempt has been made to review the emerging results on systematics, biogeography, and evolutionary relationships of most groups living in the Skadar Lake.

The high degree of species richness and endemism in freshwater gasptropods in the Skadar Lake has already been pointed out by Glöer & Pešić, and regarding these results, Glöer & Pešić stated that the Skadar Lake is an ancient lake. This is the first study with attempt to give a new insight into actual degree of biodiversity and endemism of Skadar Lake biota. Although the Skadar Lake is a younger one, his importance for evolutionary research should not be underestimated.

The adjusted rate of endemicity is estimated at 5.8 % for Animalia. The most endemic biotic groups are amphipods, snails, fishes and ostracodes. In term of endemic diversity the Skadar Lake is relatively poor compared with the ancient Ohrid Lake with his 212 known endemic species and rate of endemicity estimated at 34% for Animalia.

Due to its importance of the region, the Montenegrin part of the lake was proclaimed a National Park in 1987, whereas the unique features of the lake resulted in the classification of the Skadar as a wetland site of international significance according to the RAMSAR Convention (1995).

VARIATION OF PHARYNGEAL STRUCTURES IN SYMPATRIC SPECIES PAIRS AND ALLOPATRIC POPULATIONS OF THE ROCK-DWELLING CICHLID GENUS *TROPHEUS*

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Lake Tanganyika harbors the oldest and most diverse species flock of cichlid fishes, well known for studies of explosive speciation and adaptive radiation. The study species *Tropheus* consists of six nominal species all of which live in the upper littoral zone in various kinds of rocky habitats. Over 120 distinctly colored populations are known. In some regions more than one sister species lives in sympatry with another *Tropheus*. This study addresses character divergence of particular viscerocranial bones with known relevance for trophic specialization in allopatric populations and in sympatric populations coexisting with a sister species. Three sympatric populations of *T. moorii* and *T. polli* were analyzed in comparison to two allopatric *Tropheus* populations and one outgroup. Preparation of the selected bones (dentary, angular, premaxillary, quadrate, preopercle and pharyngeal teeth) includes enzymatic disarticulation of the heads, staining of the bones with alizarine red, and

applying a landmark system for geometric morphometric analysis on the images taken. A principle component analysis (PCA) is carried out based on partial warp and uniform component scores. To state possible variations between the three populations a canonical variate analysis (CVA) is performed. Finally, pairwise comparisons based on the CVA-results, are obtained.

While previous studies of allopatric *T. moorii* populations found a significant difference in both views of the dentary, none of the allopatric populations in this study showed any difference. It has also been shown that sympatric and allopatric populations of *T. moorii* are distinguishable via proportions of the eyeregion. To demonstrate potential differences among populations of *T. moorii*, this study focuses on the underlying bones of the eyeregion. Another objective of this work, which is still in progress, is to exemplify possible disparities between the males' and the mouthbrooding females' digestive part of the viscerocranium.

TOWARDS A COMPREHENSIVE EVOLUTIONARY HISTORY OF THE ENDEMIC COTTOID SPECIES FLOCK FROM LAKE BAIKAL (SIBERIA)

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Lake Baikal (East Siberia), the oldest ancient freshwater lake on the globe harbors several of the most species rich vertebrate and invertebrate

species flocks. One of its endemic species flock are the so-called sculpin fishes (cottoidae, Scorpaeniformes). In the context of the MOLARCH project we intended to assess the evolutionary history of cottoid populations from shallow, deep and pelagic habitats, in order to compare the evolution of populations in stable habitats (deep waters) versus unstable (or less stable) ones (shallow waters).

Recent data suggested that the Baikalian cottoids were a monophyletic species assemblage. However, our sampling included several specimens per species, and whenever possible conspecifics from different lake basins. As a consequence the monophyly of the group and of several genera and species were put into question. Therefore the first step of the study was to establish a strong molecular phylogeny of the cottoids before choosing the target species for our population study.

To infer the molecular phylogeny of these fishes we used the maximum likelihood and Bayesian approach on four nuclear fragments (ITS 1/ 5.8s rRNA/ ITS2 [1196bp], the D1-D2 region of the 28s rRNA [1046bp], the S7intron1 [540bp] and the S7intron2 [780bp]) and two mitochondrial markers (DLoop [820bp] and CO1 [1550bp]). The dataset consisted of 125 specimens representing 29 out of the 33 described cottoid species. The results show that some species and genera appear to be paraphyletic, a finding that could possibly be due to convergent morphological features, indicating that several cryptic species and genera were not yet detected.

For the population study, species that were representing undoubtedly monophyletic groups on both morphological and molecular grounds were chosen. The shallow water species (*Leocottus kesslerii*, *Paracottus knerii* and *Batrachocottus baicalensis*) and the pelagic species (*Comephorus dybowski*) display none (or weak) genetic structure compared to the deepwater ones (*Abyssocottus korotneffi*, *Batrachocottus multiradiatus*,

Limnocottus godlewskii). Furthermore the shallow water populations display over time a constant population size, which is not the case of the deep water ones. These results contradict the expectation that in stable environment population should be stable too.

COMPLETE LINEAGE SEGREGATION IN OSTRACODS (CRUSTACEA, OSTRACODA) FROM LAKE TANGANYIKA (AFRICA).

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Ostracods are small, bivalved crustaceans that abound in both freshwater and marine environments. Almost 25% of all recent non-marine species occur in ancient lakes, most of these being endemic to their respective lakes (Martens et al., 2008). About 100 species have thus far been described from Lake Tanganyika, of which the *Cyprideis* species flock is a prominent representative.

During the ESF project MOLARCH, several populations of species of the genus *Romecytheridea* were screened for mitochondrial 16S and nuclear LSU. Whereas there were no intraspecific differences in the LSU sequences, the phylogenetic reconstructions using 16S showed that all investigated populations along the southern part of the lake were fully segregated from each other. Some of these have meanwhile been described as new species. It thus appears that at least in some of the so-called species of the *Cyprideis* species flock, several cryptic species occur.

**PLANORBID STEINHEIM SNAILS REVIEWED:
EVOLUTIONARY TRIGGERS IN A FOSSIL, LONG-LIVED LAKE
(MIOCENE, S' GERMANY)**

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Studies about the Miocene snails from the Steinheim Basin are reviewed with respect to lake development and factors controlling the endemic evolution of the planorbid genus *Gyraulus* in a fossil, long-lived lake. Open questions will be raised and perspectives for further studies will be discussed in a broad context.

The Steinheim Basin is a fossil lake that was formed by a meteorite impact ca. 14 Million years ago (Late Badenian, Miocene). The crater reveals a diameter of 3,5 km and contains ca. 30-40 m thick sediments. The duration of the lake is a matter of debate and estimates vary between a few thousands and more than one Million years. This lake comprises a wealthy fauna of aquatic and reworked terrestrial snails. The most impressive group are endemic planorbids that occur in rock-forming quantities.

In 1863, only four years after the publication of Darwin's "Origin of Species", the palaeontologist Franz Hilgendorf finished a dissertation about these planorbid snails. Three years later, he presented a phylogenetic tree, which today is seen as the oldest phylogenetic tree of fossils and the oldest fossil evidence for Darwin's theory of descendance. Despite its scientific importance, the reason for the endemic evolution is still unclear. The following triggers for speciation have been discussed so

far: Water chemistry changes due to lake water evaporation, specialisation to smallest habitats in a long-lived lake, high and changing ecological pressure, and parasitism.

WAS THE EXTINCT QUATERNARY MEGA-LAKE PALAEO- MAKGADIKGADI (KALAHARI, BOTSWANA) A CENTRE OF MAJOR EVOLUTIONARY RADIATIONS?

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It has recently been proposed that the southern African mega-lake palaeo-Makgadikgadi once hosted a rapidly evolving fish species radiation, comparable in morphological diversity to that in the extant African Great Lakes. While this idea is supported by genetic and morphological analyses of modern southern African riverine cichlids, it is not without contradiction. Considering state of the art of palaeolimnological research on Lake palaeo-Makgadikgadi it becomes evident that on the one hand we are far from understanding physical, chemical and biological properties of this palaeohydrological system. On the other hand, when the regional geological setting and the geomorphology of the relevant depressions in the Kalahari are

considered, it has to be concluded that Lake palaeo-Makgadikgadi represented an endorheic lacustrine system containing a brackish water environment. The modern cichlids of southern African rivers, however, are typical freshwater species which cannot cope with increased salinity. In order to solve the riddle we have started to reconstruct the palaeolimnology of Lake palaeo-Makgadikgadi in more detail. The geomorphology of the palaeolake-system was re-examined and fluvial and lacustrine sediments were investigated with respect to fossil remains of aquatic organisms. Preliminary results indicate that at least during the last ca. 45 ka BP Lake palaeo-Makgadikgadi was much smaller than previously assumed, having contained a brackish to saline environment. Corresponding data obtained through the examination of fossil molluscs and ostracodes respectively from outcrops of the Boteti River valley and from palaeo-beach deposits of Lekubu Island (Sua Pan) are presented and discussed.

GASTROPOD DIVERSIFICATION IN THE ANCIENT LAKES OF SULAWESI: A KEY ROLE FOR TROPHIC MORPHOLOGY

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Key modifications in morphological characters related to feeding, such as beak size and shape in the Galapagos finches or variations in the pharyngeal jaw of cichlids, are a common factor in many adaptive radiations. The gastropod species flocks of *Tylomelania*

(Caenogastropoda: Pachychilidae) in the two ancient lake systems of the Indonesian island Sulawesi are no exception here. The lacustrine species exhibit a wide variety in trophic morphology, i.e. radula form, which is almost entirely lacking in riverine species. This coincides with all lake species being specialists in the sense that each species only occurs on one type of substrate such as hard (rocks, wood) vs. soft substrate (mud). Frequently the radula is species specific. Vastly different radulae are often found in species dwelling on different substrates or also in species occurring sympatric on the same substrate. This simple basic pattern becomes considerably more complicated in several taxa, though. Species dwelling on both hard substrates in particular are frequently polymorphic in radula form. While a correlation between radula type and substrate could be established in some cases, this is by no means universal, even within the same species. Potential explanations for these findings range from incipient ecology-driven speciation to phenotypic plasticity and thus suggest a far more complicated role of radula evolution in the adaptive radiation of *Tylomelania* than previously assumed.

PHYLOGENETIC RELATIONSHIPS OF LAKE OHRID SURIRELLACEAE TAXA

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Lakes of tectonic basins of the world are well known for remarkable biodiversity and endemism of freshwater aquatic flora and fauna. The ancient lakes Ohrid and Prespa have features that provide a valuable opportunity to test hypotheses about species distributions. Diatoms are unicellular, photosynthetic algae found in virtually every habitat with sufficient water and sunlight. Their abundance, widespread distribution, and resilient siliceous cell walls make them an ideal tool for a wide range of applications both as fossils and living organisms.

The diatom family Surirellaceae consists of seven genera that exhibit diverse valve morphologies and are ideal for testing diatom speciation hypotheses. The most species-rich genus, *Surirella* (>650 species), exhibits a high degree of endemism, especially within the Indonesian archipelago and the ancient lakes Baikal, Tanganyika, and Ohrid. Although total Surirellaceae diversity within Lakes Ohrid and Prespa is unknown, it has been proposed that at least 26 species from this family have originated within the basin. Do these species represent a lineage that originated within the lake and therefore are each other's closest relatives or do multiple independent introductions account for their diversity? As part of a wider study to reconstruct the phylogenetic relationships of the order Surirellales, DNA sequence data from the nuclear, chloroplast and mitochondrial genomes of several putative endemic Surirelloid taxa was collected and included in a global phylogeny. Results will be discussed along with possible implications for the Ubiquitous Dispersal Hypothesis (UDH).

TROPHIC STATUS OF LAKES OHRID AND PRESPA IN ACCORDANCE TO PHYSICAL, CHEMICAL AND BIOLOGICAL PARAMETERS

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The lake systems are highly dependent on their environment, i.e. on the physical, chemical and biological variables of the watershed area. The humans' influence and the changes that are made due to humans' activities, as well as the natural processes that occur in the watershed area contribute to the increased flow of nutrients which can result in a change of the trophic state of the littoral region which certainly has a great influence and it affects the pelagic zone of the water ecosystems.

Lakes Ohrid and Prespa are the largest and most important natural water ecosystems in the Republic of Macedonia. They belong to the Desaretian group of lakes and with their natural rarities and endemic species which live in them, they are classified within the group of the most important and the oldest fresh-water systems worldwide. This is the main reason for the increased interest of a vast number of scientists, coming from our country as well as from different prestigious world scientific institutions, for these two lakes to be the most desirable location for realization and development of different scientific studies.

However, the contemporary way of life and the rapid technological development of these regions affect the quality of the waters of these water ecosystems. That is how the goal of this study has been revealed as well. In other words the aim is to present the trophic status of these

two lakes in accordance to the level of their production, or to be exact, to present the intensity of the process of eutrophication.

According to the researches which were conducted in the period 2004 – 2006, a comparison of the trophic status of the Lakes Ohrid and Prespa in their pelagic zone, in conformity of the nutrients' concentrations and transparency as physical-chemical parameters and the contains of chlorophyll a, as a biological consideration. The vertical profile of the Lake Ohrid includes 10 depths while for the Lake Prespa there is a profile with four depths. With the implementation of the Carlson's method of determination of the trophic condition of the surface water, OECD classification system of the lakes in the moderate climate zone and in accordance to the classification system developed on average summer values of the trophic parameters of the surface levels of the lakes according to Forsberg & Ryding and Nurnberg, one can easily attain the entire image of the trophic state of the lakes.

From the gained results it can be concluded that the waters of the Lake Ohrid, in accordance to the three classification systems, is to be considered as an oligotrophic lake. Unfortunately, the results from the analysis of the waters of the Lake Prespa are indicating that a strong process of eutrophication is occurring in this ecosystem, i.e. it is considered as a lake with mesotrophic character.

CONCORDANT PATTERNS OF GASTROPOD EVOLUTION IN LAKE MALAWI

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Studies on ancient lakes considerably contributed to our understanding of the driving forces behind evolutionary processes. As such, the presence (or absence) of, e.g., competitors and predators may influence specific evolutionary histories, whereas abiotic factors such as the environmental stability of a lake may affect the evolution of a wide range of its taxa. Here we present the results of the analyses of species from two gastropod genera from Lake Malawi that revealed concordant evolutionary patterns indicating general driving forces: (1) Fossil specimens of *Lanistes* (Ampullariidae) and *Bellamya* (Viviparidae) from Pliocene beds in Northern Malawi resemble in part the modern endemic species in the lake – seemingly pointing towards ancient radiations, which started more than 2 million years ago. However, integrating fossil data with findings from molecular analyses reveals a more complex, yet rather similar pattern for both endemic groups: Based on trait-specific molecular clock analyses, we found that the accumulation of the extant molecular diversity in each taxon did not start in the Pliocene but at least 1 million years later in the Pleistocene from single lineages. This indicates that – except for two founding lineages – the Pliocene species of both groups went extinct. We argue that severe changes in Malawi's lake level during the Pleistocene

offer an explanation for the concordant pattern. This view is also supported by demographic analyses using a coalescent framework, which indicates a significant expansion of effective population size after the onset of molecular diversification. (2) In contrast to previous reports, all specimens of the genera *Lanistes* and *Bellamya* found in the Malawi Rift are endemic and form monophyletic and – in a rift-wide phylogenetic context – highly distinct groups. Shell similarities to other African species of these genera appear to be cases of parallel evolution or plesiomorphy. This 'rift-endemism' stands in contrast to reports from other ancient lakes where endemism is restricted to the lake proper.

Future studies will show whether these concordant evolutionary patterns can also be found in other endemic taxa of Lake Malawi. Thus, our study is a plea for more detailed analyses of the invertebrates of this famous rift-lake in order to shed more light on the timing as well as on general forces driving the evolution of Malawi's endemic fauna.

First Data on Biology of Shallow-water Spongillidae from Lake Baikal

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Lake Baikal is inhabited by representatives of two families of sponges: endemic Lubomirskiidae and cosmopolitan Spongillidae. It is generally

accepted that the sponges of the family Lubomirskiidae descended from Spongillidae. Life cycle of Lubomirskiidae changed greatly during the evolution process. Life spans increased and gemmulation was lost in the stable Baikal environment. Spongillidae from warmwater bays of Baikal (genera *Spongilla*, *Eunapus*, *Ephydatia*) retain gemmulation in their life cycles. *Trochospongilla* is the only Spongillidae genus living in open Baikal waters. These sponges do not produce gemmules. This genus is considered to be the possible ancestor of Lubomirskiidae.

Baikal *Trochospongilla* biology was investigated using samples from the Berezovy test-side (South Baikal), which is a typical shallow-water of open Baikal. The samples were determined at genus level due to the lack of gemmules. *Trochospongilla* sp. inhabits the depth range of 1 to 8 m. The sponge is presented in the samples taken throughout the year. This is the evidence that Baikal *Trochospongilla* sp. (as distinct of Palaearctic one) survives the winter period. A histological approach was used in order to investigate the sexual reproduction of the Spongillidae in Baikal. The embryos at the stage of cleavage were presented in the February samples. The oocytes at the late stage of vitellogenesis, the embryos at the stage of cleavage, the larvae and the spermatogenesis were observed in June samples. It proves *Trochospongilla* sp. is able to sexually reproduce in open Baikal.

Parallel and independent reduction of gemmule stage in the life cycles of ancestral forms of Ochridaspongia and Lubomirskiidae should be considered as one of the most interesting and fundamental problems of freshwater sponge biology. This is the focus of our future investigation.

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THE ANTHROPOGENIC FACTOR AND THE QUALITY OF THE WATER FROM GOLEMA RIVER AND THE LITTORAL OF ITS DELTA

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The second-largest lake in the Republic of Macedonia is the Prespa Lake. The total surface of the lake is 313.6 squared kilometers, 190 km² of which belong to the Republic of Macedonia, 84.8 km² to the Republic of Greece and 38.8 km² belong to the Republic of Albania.

There is no surface connection between the Prespa and Lake Ohrid, but the waters of Lake Prespa leaks to the Lake Ohrid through an underground flow. That is why the goal for prevention of the Prespa Lake is twofold, i.e. as a water ecosystem and as a source of water for the Lake Ohrid.

There are no underwater sources registered in the Prespa Lake, and even if they do exist their influence is with no importance. This lake is usually supplied with water from surface inflows, the majority of which are located in the Macedonian part. The most important tributary to the lake is the inflow of water from the Golema (Big) River which is a valley river and flows from north. During the summer period the waters of this river are used for irrigation of the apple plantations – famous for the Prespa region. As a result in the lower flow of the river there is a minimal quantity of water – mainly communal and industrial waste water.

The quality of the water of River Golema and the littoral zone Asamati, at the spot where the river water flows into the lake is going to be presented through physical and chemical parameters.

The aim of this study is to present the quality of the water in the analyzed localities by tracking of the biochemical consumption of oxygen, the consumption of KMnO_4 as well as the concentrations of the total nitrogen and total phosphorus. The samples are collected with seasonal dynamic in the period winter 2008 – spring 2009. For definition of the level of encumbrance of the water from the specific analyzed spots are used the OECD – regulations and the Regulation of Waters of the Republic of Macedonia.

**WAS THERE AN ANCIENT LAKE IN THE ARAX VALLEY (ARMENIA)?
– EVIDENCE FROM A PHYLOGEOGRAPHICAL ANALYSIS OF
THEODOXUS SPP. (GASTROPODA: NERITIDAE)**

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A lake of long existence can be extant (ancient lake) or fossil (palaeolake), and it has been suggested that palaeolakes existed in the past when hydrological conditions were different. Even though these palaeolakes may have been subject to desiccation, their water sources (e.g., springs or rivers) may have continued to exist until today and could thus have served as reservoir for the former lake fauna. The Mexican desert spring pools of Cuatro Ciénegas, e.g., constitute such a case. On the territory of present Armenia in the Caucasus region, the existence of several palaeolakes was suggested as well. One of these lakes was

supposedly situated in today's Arax Valley, which also harbors the karstic spring system Aiger-Lich. This limnocrene system supposedly is of Pliocene age and could thus constitute an extant source of the palaeolake.

In the present study we test this hypothesis by studying the phylogenetic relationships and the age of origin of gastropod populations of the genus *Theodoxus* (Neritidae). Previous studies suggested that the evolution of this group is closely associated with palaeolakes.

Therefore, fragments of the mitochondrial cytochrome c oxidase I gene (COI) of specimens from the Aiger-Lich spring system, the Hrazdan River near Yerevan and from the Caspian Sea were analyzed together with previously published *Theodoxus* COI sequences. The reconstructed phylogeny revealed a distinct clade (*T. pallasii* s. l.) with an Armenian subclade (Aiger Lich and Arax catchment) supporting a close relationship of Caspian and central Caucasian malacofaunas. Moreover, our results support the hypothesis of existence of a transient connection between ancient Caspian Sea and the Armenian hypothetical palaeolake in the Arax Valley. *Theodoxus* from the Arax Valley could have originated in this hypothetical lake and the recent population could be derived from a common ancestor in the area. A molecular clock approach with trait-specific clock rate was used in order to estimate the time of origin and diversification of the *T. pallasii* s. l. clade. This lineage diversified in the time frame of late Pliocene to early Pleistocene. Contemporary palaeogeographical studies show that in the middle to late Pliocene on the territory of present Armenia, a large freshwater basin existed in the Arax valley with a connection to the brackish Achkagylian Basin in the Caspian depression. The pattern of segregation we found in mitochondrial lineages of recent Caucasian and Caspian *Theodoxus* populations is in concordance with this concept.

POSSIBLE GENETIC MECHANISMS OF ACCELERATED DIVERSIFICATION IN SPECIES FLOCKS

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Baikalian species flocks evolved in a dramatically changing environment. The eco-system fluctuations occurred due to global climate changes and fast dramatic tectonic processes. As a result, the lake level and food fluxes from the shores changed quickly and with high amplitude. It is more or less commonly accepted, that such conditions would inhibit niche splitting and speciation processes, promoting the evolution of few mostly generalist species. This contrasts with striking discrepancy with the very high diversity of sister species observed in the ancient lakes.

Here we use individual-based computer simulations for detailed examination of the above-mentioned theoretical prediction and show that it is only true in the case if the adaptive response of a species to changing environment is slower than the environmental fluctuation. If there is a very high adaptive potential within a species, niche splitting is still possible even under rapidly fluctuating conditions. The theoretical study allowed us to make some predictions on the pattern of molecular diversity in a neutral marker and to estimate the amount of sequence data required for testing this hypothesis.

We show that for relatively recent speciation events in species flocks, the diversification time estimated as the difference between the species division time according to IM model and the coalescent tMRCA estimate must be shorter than the period of the most dramatic environmental

fluctuations observed in paleo-record in Lake Baikal. This turns out to be the case for all members of young species flocks tested in Lake Baikal. Unfortunately, to date it was not possible to test another prediction from the model: the speed of speciation in groups consisting of few species must be lower than the speed of eco-system changes.

Very high speciation speeds must involve unusual genetic mechanisms promoting reproductive isolation. We hypothesize that such a "catalysis of speciation" may play an important role in the organisation of genome and report an example of molecular evolution in an intron which may cause partial recombination arrest due to accumulation of mostly point mutations.

At species diversity level, the hypothesis predicts that the ecosystems like that of Lake Baikal must be enriched by highly diverse organism groups as well as by mono-typic taxa of relatively high rank at the expense of taxa consisting of few species, which are typical of more stable ecosystems.

**LIMPETS OF THE GENUS *ACROLOXUS* (GASTROPODA:
PULMONATA: ACROLOXIDAE) IN LAKE BAIKAL: TAXONOMY,
SHELL MORPHOLOGY, DISTRIBUTION, ORIGIN**

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According to published data, the family Acroloxidae in Lake Baikal radiated extensively and today numbers 25 species belonging to three endemic genera. So far, a single species of the Holarctic genus *Acroloxus* – *A. baicalensis* has been known in the Baikal fauna. The species was first described from the Chivyrkui Bay as East Siberian subspecies of the widespread *A. lacustris* and was later treated as a regular species.

Besides Chivyrkui Bay of Baikal and the Lower Yenisei River (Dudinka settl. vicinity), *A. baicalensis* inhabits lakes Ivan and Arachlei, the Angara River basin and small lakes near the south-east Baikal coast. Limpets morphologically resembling *A. baicalensis* occur on slow rivers in the Southern Russian Far East together with *A. likharevi*. The distribution range of *A. baicalensis* is probably limited by the Yenisei River to the west and the Pacific Ocean shores to the east.

The limpet fauna of Lake Baikal is rather well-studied. In 2008 we found two new species for the Baikal fauna in Kotovo Bay of the Chivyrkui Inlet – *A. orientalis* and *Acroloxus* sp. nov. *A. orientalis* was described from the Southern Russian Far East and has recently been recognized in the Baikal basin for the first time. Thus, the range of *A. orientalis* covers the territory from Baikal to Sakhalin Island and is generally concordant with that of *A. baicalensis*. Both species are ecologically similar and inhabit the macrophytic vegetation of slow rivers and big lakes. *A. baicalensis*, however, sometimes lives on hard substrate as well. In Lake Baikal, these limpet species were collected sympatrically from the aquatic plant *Elodea canadensis* at a depth of 1.5 m.

The shell morphology of the Baikal *Acroloxus* species collected in Chivyrkui Bay was studied using SEM. Interspecific differences in protoconch microsculpture between *A. baicalensis* and *A. orientalis* were not found. Both species are characterized by a horn-shaped protoconch with a less strong reticular sculpture than littoral endemic acroloxids. The back slope of the protoconch has both radial and concentric ribs. On the front slope a very slight reticular sculpture is substituted by dots. Initial plates of *A. baicalensis* and *A. orientalis* overlap each other in its dimensions: for the first species the length is 0.2–0.24 mm, and the width 0.13–0.16 mm; for the second one correspondingly 0.21–0.27 mm and 0.15–0.19 mm. *Acroloxus* sp. nov. differs from the two others significantly by its cap-shaped protoconch with pitted microsculpture. Note, the protoconch bearing rows of pits are known from the single abyssal Baikal limpet – *Pseudancylostrem frolikhae*.

Baikalian *A. baicalensis* and *A. orientalis* are characterized by a pronounced variability in their shell morphology. There are many specimens with intermediate shell shape, which may be evidence of cross-breeding. Typical *A. orientalis* are characterized by a flat shell (shell

height (H) to aperture width (W) ratio ≤ 0.54), deep undertop concavity, evenly convex right slope and rounded front and back aperture edges. *Acroloxus baicalensis* has a higher shell ($H/W \geq 0.56$) with slight undertop concavity and a nearly straight right slope, and an aperture with wider frontal edge. There are some differences in shell morphology between Baikalian and Far East *A. orientalis* specimens as well. Baikalian specimens have a shell apex located near the left aperture edge (wL) to 0.25–0.30 of its width; apex distance from frontal aperture edge (La) is 0.69–0.73 of its length (L). The shell apex of the Far Eastern *A. orientalis* specimens is close to the middle ($wL/W=0.4–0.45$, $La/L=0.58–0.61$). In addition, the shell apex of *A. baicalensis* from Chivyrkui Bay is closer to the frontal aperture edge ($La/L=0.71–0.72$) in comparison with specimen from Yenisei R. ($La/L=0.79$).

Given the new data we assume that *A. baicalensis* and *A. orientalis* are closely related allopatric and somewhat cryptic species with overlapping distributions in the eastern littoral of Baikal. Both species possibly form the sister group to *Acroloxus* sp. nov. that should be described formally. Future studies using appropriate genetic markers should address the phylogenetic and phylogeographic status of the populations of *Acroloxus* spp. of Lake Baikal and surrounding watersheds.

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ECOLOGICAL ADAPTIVE EVOLUTION OF ENDEMIC COTTOID FISHES (COTTOIDEI) FROM LAKE BAIKAL

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The endemic ichthyofauna of Lake Baikal consists of sculpins which number 33 species in 11 genera and 3 families. The species range from inshore areas to the deepest parts (1700 m) of the lake. Cottoid endemic fishes make up 54% of the total ichthyofauna and 100% of the abyssal fishes in Lake Baikal. Sculpins from Lake Baikal are an original example of endemic species flocks, like in the other large ancient lakes. The presence of families and a subfamily with different level of adaptation to Baikal conditions and the absence of transitional forms among them, suggests a different period at which the Cottoidei ancestors became established in the lake.

Ecological adaptive evolution of cottoid fishes in Lake Baikal developed in four strategies:

1. Ancestral littoral species occupied a deepwater zone and formed benthic abyssal freshwater cottoid fishes;
2. Deepwater species re-colonized a coastal zone and created secondarily littoral forms;
3. Fishes adapted to the unique biotopes and formed local endemics;
4. Cottoid fishes expanded the pelagic zone and developed pelagic forms.

1. Ecological evolution for deepwater zone occupation is a leading feature in Baikal Cottoids. Twenty-two species or approximately 70% of all cottoid species in the lake are deepwater fishes. Maximum number of deepwater Cottoid species occurs at 150-500 meters. The deep-water zone (more

than 1000 meters) is inhabited by the same number of cottoid species as the coastal zone. It is an interesting paradox of the vertical distribution. Deep-water cottoid fishes are mostly present as eurybathic species (50%), inhabiting a wide range of depths. For example, *Limnocottus pallidus* lives at depths of 150 - 900 meters and *Abyssocottus korotneffi* – from 400 to 1700 meters.

The only “stenobathic” species is *Neocottus werestschagini*, discovered at a depths of 900-1400 (nine-hundred – fourteen hundreds) meters. Expansion of fishes from the coastal zone to the abyssal very often is associated with a speciation inside one genus. For example, *Batrachocottus baicalensis* inhabits the littoral, *B. multiradiatus* lives deeper, *B. talievi* even deeper, and finally *B. nikolskii* occurs in the abyssal zone.

Expansion in the deep-water zone is impossible without adaptation to the high hydrostatic pressure. Our experiments show that deep-water species can withstand pressures of 270 atm, which is significantly greater than those found at the maximal depth of the lake (170 atm) and comparable to the pressure at oceanic depths. Only deep-water marine Cottoidei can resist this pressure.

All deep-water cottoid species have large eggs – 2.0 - 3.7 mm in diameter, and benthic larvae.

2. The next pattern of the ecological evolution of benthic cottoid fishes is re-colonization of the coastal zone by the deep-water species, which result in the secondary littoral forms. This direction is not very developed in Baikal cottoids. Only four species of the genus *Procottus* followed this direction of evolution. These species have a very high level of the morphological specialization, typical for all deep-water cottoid fishes in Lake Baikal.

3. Third direction of ecological evolution is adaptation to unique biotopes and formation of local endemic forms. Many unusual, unique biotopes were recently found on the bottom of Lake Baikal. They develop near the bottom hydrothermal vents. So far, only the fauna of cottoid fishes in the area of hydrothermal vent located in the Frolikha bay has been studied. This hydrothermal vent occupies an area of only 600 square meters. However, unique conditions surrounded the vent (bacterial mats, chemosynthesizing algae and bacteria), created a local endemic *Neocottus termalis*. This species lives only in the thermal vent area.

Such a trend in speciation is not characteristics of lakes. It is most common in oceanic systems.

4. The next pattern of ecological evolution is expansion of the primarily benthic species into the pelagic zone, resulting in the pelagic forms. This type of ecological evolution is not typical for cottoid fishes and was only found in endemics of Lake Baikal.

Five species from Baikal Cottoidei are pelagic. Three of these species belong to the endemic genus *Cottocomephorus*. They live at depths of 20 - 500 meters and are still connected to the bottom. They stay on the bottom for 30-50% of the day and the rest of time they spend swimming in mid-water. Zooplankton makes up 60-100% of this fish's diet, but benthic organisms are also found in their food (amphipods, mollusks, caddis flies and ostracods). Pelagic species reproduce in the same way as benthic cottids, laying eggs on the lower surface of rocks and stones. Their larvae are pelagic.

The pair of species which form the endemic pelagic family *Comephoridae* has a number of deep-pelagic adaptations. They live in mid-water at depths of 100-1700 meters. All stages of ontogenesis occur in the water column, and embryonic development is specialized, leading to internal fertilization and ovoviviparity. Their larvae are also pelagic. Their diet is

pelagic zooplankton and these fishes show vertical migration when feeding. Comephoridae are the most specialized and abundant fishes in Lake Baikal.

Endemic fauna of cottoid fishes of Lake Baikal is extremely diverse ecologically. As a result of ecological evolution secondarily pelagic ovoviviparous forms developed from benthic cottoid ancestors. Development of strict specialization for water habitats is a unique phenomenon and normally it is not characteristic of marine Cottidae. Evolution of deep water species is a tendency of the ecological evolution observed in marine Cottidae.

Adaptive evolution of endemic cottoid fishes from Lake Baikal shows their differences to other freshwater lakes and supports the idea that Lake Baikal is an oceanic type ecosystem.

**DOES THE SIBERIAN AMNICOLID GASTROPOD GENUS
KOLHYMAMNICOLA STAROBOGATOV ET BUDNIKOVA
REPRESENT THE SISTER GROUP OF BAICAILIIDAE ENDEMIC TO
LAKE BAIKAL?**

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Although the malacofauna of Lake Baikal has been studied for more than a century origins of the endemic groups remain obscure. With

respect to baicaliid gastropods the Ponto-Caspian Pyrgulidae had been suspected of being closely related but more recently it was suggested that Amnicolidae or Bithyniidae may represent the sister group. The latter conclusions were based on the comparative study of the two genera *Marstoniopsis* (Amnicolidae) and *Bithynia* (Bithyniidae). In order to test the amnicolid origin hypothesis we considered East Siberian taxa of this family, whose species do not occur in Lake Baikal but in its proximity. Morphological studies demonstrated that species of the genus *Kolhymamnicola* show traits which clearly distinguish them from other amnicolids and which some authors considered a distinct family Kolhymamnicolidae. Recently more detailed morphological and anatomical studies on four *Kolhymamnicola* species were conducted with the aim of obtaining a character set to be used for a comparison with Baicaliidae. All of the investigated *Kolhymamnicola* species are characterized by small and smooth shells of up to 5 mm in length. The protoconchs are sculptured by weak spiral lirae and terminate after 1.25-1.5 whorls at a diameter in a range of 0.5-0.8 mm. The non-spiral of the protoconch is 0.16-0.19 mm in width. A similar protoconch sculpture can be found e.g. in the baicaliid *Teratobaikalia nana* which is the smallest species of the family. Operculum of *Kolhymamnicola* species are paucispiral and thin and thus resemble those of most baicaliids. The ctenidium in *Kolhymamnicola* has 12-14 leaflets and is short whilst it is long in baicaliids where the ctenidia consist of 50-150 leaflets. The osphradium in *Kolhymamnicola* is comparatively wide situated in the middle part of the mantle cavity. Osphradia are relatively narrow in baicaliids and are found in part of the front of the mantle cavity. The four examined *Kolhymamnicola* species differ clearly from baicaliids with respect to radular teeth but resemble amnicolids.

Kolhymamnicola species live in shallow water bodies with few currents, often attached to water plants. They are distributed over the vast territory of East Siberia, from the Lena River system to the shores of the Pacific Ocean including Kamchatka, Sakhalin, Kuril Archipelago (extreme northern and southern islands) and one species was recently detected in western Alaska. Also quite recently a species of *Kolhymamnicola* was found in one of the lakes located in the Baikal Rift Zone. The distribution of *Kolhymamnicola* is quite similar to that of some other zootaxa having relatives among Lake Baikal endemics such as the planarian *Bdellocephala*, the gastropod *Choanomphalus* (*Vitreoplanorbis*), the polychaete *Manayunkia* “*baicalensis*” and fish species of the Cottoidei. It has been speculated that these biogeographical patterns have their common root in the Late Cretaceous to Palaeogene Zabaikalie lake-river system south and east of the area where the East Siberian Rift System triggered the Eocene-Oligocene formation of the initial Lake Baikal. Fossils amnicolids, however, have never been described in lacustrine sediments of the East Siberian Rift System and its Baikal Rift Zone. It can be speculated that fossil amnicolids have not yet been differentiated from the many Late Cretaceous to Oligocene rissoidan gastropods of northern Asia described in literature. Future investigations should focus molecular studies on *Kolhymamnicola* species and re-examination of fossil material. We would like to thank RSF, grant FEBRAS N 09–III–A–06–181 and DFG for financial support.

DIRECT EVIDENCE OF SOME POSSIBLE ECOLOGICAL BARRIERS IN AN ANCIENT LAKE (OHRIDSKO EZERO, MACEDONIA)

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As a rule, ancient – and even some younger – lakes contain flocks of species, generally regarded as resulting from the intralacustrine speciation. In many cases, the intralacustrine speciation is explicitly supposed to be sympatric, since no barriers can be observed to cause division of populations before their differentiation. The bottom of Ohridsko ezero has been presented as a system of more or less homogeneous concentric rings, allowing the animals movement and gene flow all around the lake, as well as parapatric contacts in the vertical sense.

In fact, these rings are fragmented into islands, divided by physically or biotically different belts. Autecological investigations are necessary to show to what degree these belts are passable for small invertebrates, but some of them are certainly impassable for some of them.

The differentiation of the shallow littoral into areas of sand, of gravel, of rock, barren or overgrown by *Phragmites* or by submerged macrophytes (*Potamogeton*, *Vallisneria*, *Myriophyllum*, *Ceratophyllum*) is visible from the shore. Areas of sublacustrine springs are also ecologically specific again. At 5-20 m depth a quasi continuous belt of Characeae has developed; it is physically very specific, highly productive, and therefore inhabited by competitively strong non-endemic animals. This probably represent an effective vertical barrier; its lower border is particularly abrupt. The sublittoral are areas of silt, locally mixed with clam shells and

of flat barren rocks. The *Dreissena* zone is in fact dense colonies of clams separated by silt or barren rock. Clam colonies are densely inhabited with vagile animals which might find it difficult to cross the barren bottom between them.

FUXIAN HU (YUNNAN, CHINA), A LITTLE KNOWN ANCIENT LAKE

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Fuxian Hu is one of the lakes in the Yunnan high plateau. They are assumed to be a few million years old and Fuxian is over 150 m deep. The well investigated Fuxian fish fauna consists of 25 indigenous species but only three pairs and a triplet are congeneric, indicating the possibility of intralacustrine speciation; 12 species or subspecies are endemic. At least three Tubificinae species and a similar number of Ostracoda must at present be regarded as endemic. Unfortunately, Gastropoda were not systematically investigated. Fuxian Hu was only explicitly mentioned as an example of an 'ancient lake' recently.

Our sampling showed some endemic Amphipoda Anisogammaridae. The endemic genus *Fuxigammarus* is represented by three oligo-stenothermal species, coastal in winter, but absent from the shallows in summer. Some undescribed species from deeper waters probably belong to this genus. A molecular phylogenetic study is taking place. The rare deep-water species with spine-shaped protuberances, *Fuxiana yangi*, is a taxonomical enigma, but probably an anisogammarid.

The poverty in the biomass was a real surprise. The latter is less than 10% of that in the south-European lake of Ohrid, which shows many detailed similarities to Fuxian. Invertebrates are present in Ohrid with 10.000 indiv/m² at 30 m and over 7.000 at 50 m depth; in Fuxian, this is only 825 up to 50 m and 230 at 50-100 m depth. This poor biomass on the well aerated deep bottom icontradicts the relatively high littoral primary production and is difficult to explain. The species numbers of some groups are of the same category or moderately different in both lakes; such are Pisces, Amphipoda, Insecta, probably Ostracoda. Gastropoda are highly speciose in Ohrid, very modest in Fuxian. Turbellaria are virtually non-existent in Fuxian.

A dense belt of filamentous algae (while *Chara* is nearly absent), 14 species of introduced fish, difficulties in maintaining a sustainable fishery, and the planned increase of tourism alert us the necessity of intensifying limnological investigations and protecting this particularly fragile ecosystem. Fortunately, the local authorities noticed the conflict between the financially promising tourism and some less profitable economical branches; they limited some detrimental activities, like agriculture and use of chemicals on the lake's shore.

CHIRONOMID LARVA FAUNA (*DIPTERA: CHIRONOMIDAE*) FROM RIVER DALJAN – TRIBUTARY OF LAKE OHRID

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In the researching period from 2003 to 2003, four points on the river Daljan were considered and 12 species of chironomid fauna in larval stadium were found. Dominant species in River Daljan are represented with subfam. Orthocladinae (6 species): *Prodiamesa olivacea*, *Orthocladus saxicola*, *Psectrocladius dilatatus*, *Potthastia aedi*, *Cricotopus algarum*, *Thinemanniella flavifotceps*; subfam. Chironominae, (4 species): *Chironomus plumosus*, *C. thumi*, *Polypedilum brevianteatum*, *Paratendipes albimanus*; subfam. Tanypodinae (2 species): *Procladius choreus* and *Ablabesmya tetrasticta*. The maximum density of population *Paratendipes albimanus* occurred in June and it is 843 ind./m².

**MORPHOLOGICAL EVOLUTION OF A LAKE BAIKAL SPECIES
FLOCK: VARIABILITY OF CUTICULAR STRUCTURES IN THE GENUS
CHOANOMPHALUS (GASTROPODA: PLANORBIDAE), THEIR
TAXONOMIC AND PHYLOGENETIC SIGNIFICANCE**

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Freshwater pulmonate gastropods do not usually radiate extensively, however, there are notable exceptions e.g. in Lake Ohrid and Lake

Baikal. The genus *Choanomphalus* Gerstfeldt, 1852 is one of the most enigmatic groups within the family Planorbidae including more than 30 species assigned to 11 subgenera. The systematics of the taxon is still being debated. Two species are conventionally attributed to the genus *Gyraulus*, the European *C. (Lamorbis) riparius* and *C. (Pseudogyraulus) rossmaessleri*. Additionally, there is the Altaian *C. (P.) planoconcavus* and *C. (P.) ochoticus* from the coasts of the Okhotsk Sea. A single species -*C. hyaliniformis* of subgenus *Vitreoplanorbis*- was described from the Amur River drainage. The other 8 subgenera include 18 endemic species of Lake Baikal forming a species flock, 5 species endemic to Lake Baikal and the Angara River, and a single endemic species of Lake Hövsgöl.

In the current study, we use morphological data in order to clarify the systematics of the genus *Choanomphalus* with focus on the species of Lake Baikal. After examining stylet and radula structure using a scanning electron microscope, we divided the genus *Choanomphalus* into 4 groups:

1. species with a cone-shaped stylet 15 to 45 μm long, mainly with plicate and rectangular radular teeth – Baikal shallow water lithobiontic *C. annuliformis*, *C. gerstfeldtianus*, *C. grachevi*, *C. korotnevi*, *C. patulaeformis*, *C. schrenki*, *C. huzhirensis*, litho-psammobiontic *C. kozhovi*, abyssal *C. bathybius* and *C. planorbiformis*, as well as the European *C. riparius* and *C. rossmaessleri*;
2. species with a cone-shaped stylet 50 to 115 μm long and bifurcate radular teeth – Baikal shallow water phytobiontic *C. angulatus*, *C. valvatoides*, and psammobiontic *C. anomphalus*, Baikal-Angara litho-psammobiontic *C. amauronius* and *C. aorus*, as well as the Lake Hövsgöl *C. mongolicus*;

3. species with a nail-shaped stylet 60-120 μm long and bifurcate or rectangular radular teeth – Baikal-Angara shallow water psammobiontic *C. cryptomphalus* and lithobiontic *C. incertus* and *C. maacki*;

4. species with a cap-shaped stylet 10-12 μm long and rectangular radular teeth in lithobiontic *C. eurystomus* and bifurcate radular teeth in psammobiontic *C. microtrochus*. According to the radular teeth sizes, their number in a transverse row and radular ribbon length, each of the first 3 groups is subdivided into subgroups consisting of 2-4 related species. These groups differ in the structure of their shell morphology and anatomical characters, including the reproductive system and the kidney. Based on the available morphological and ecological data, a new hypothesis on the intrageneric relationships of the genus *Choanomphalus* s. str. has been proposed. It gives new insight into the taxonomic composition and tentative speciation trends. We provide information that complements the previously published suggestion that the common ancestor of Lake Baikal *Choanomphalus* existed about 3.5 MY ago. It also supports previous hypotheses favoring allopatric divergence in various habitats and geographical zones of the lake as the main mode of Baikal endemics speciation. One of these hypotheses suggests hybridogenic origin for some species, and the other – occurrence of deep water species in the localities of natural hydrotherm and hydrocarbon (oil) release. Future molecular studies will shed more light on the evolutionary history of *Choanomphalus* in Lake Baikal.

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MORPHOLOGICAL AND GENETIC POPULATION DIFFERENTIATION IN *ALTOLAMPROLOGUS COMPRESSICEPS*

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Lake Tanganyika is well known for its extraordinary diverse cichlid fish fauna and represents an often studied system for different speciation processes. The Lamprologini represent the most species-rich tribe in Lake Tanganyika comprising 90 species in 7 genera. Most of the lamprologine species are endemic to Lake Tanganyika and represent a monophyletic group.

Altolamprologus compressiceps is a substrate spawning, highly specialized philopatric species in rocky habitats. Here, it prefers living between boulders and seems to avoid crossing large sandy areas. Those stretches of unsuitable habitat can increase population differentiation if gene flow is interrupted.

In this study we want to investigate population differentiation in *Altolamprologus compressiceps*. We are analyzing geographically separated populations from three bays at the southern tip of Lake Tanganyika (Zambia). We will assess population differentiation and levels of gene flow among populations using nuclear DNA markers in terms of highly polymorphic microsatellite loci. In addition to the genetic markersystem, differences in the overall body shape are analyzed using geometric morphometrics.

Results will shed light on genetic and morphological population differentiation and levels of gene flow in this rather philopatric species.

CONCORDANT FEMALE MATE PREFERENCES IN THE CICHLID *TROPHEUS*

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Variance in reproductive success among males in a population, such as resulting from female mate choice, is one of the explanations for the rapid evolution of traits like the colorful body patterns of some cichlid fishes. Polygynous populations are generally able to accrue a higher variance in male reproductive success than monogamous ones, and indeed, many of the colorful cichlid species are polygynous; moreover, females visit multiple males to spawn a clutch. In contrast, one of the most variably colored cichlids, the Lake Tanganyika endemic *Tropheus moorii*, forms temporary pair bonds and females spawn with only one male per clutch. After spawning, females abandon their mates to mouthbrood their fry, which sets the males free to mate again. Theoretically, variance in male mating success can accumulate over time, if some males are selected repeatedly in succession, while others acquire few if any partners. When pairs of male *Tropheus* (population “Chimba”) were presented to several females in a row in an experimental setup, there was a significant consensus among females on the more attractive of the two males. In each of three male pairs, the same male was chosen by 5-7 consecutively tested females ($p < 0.05$ for random choice among males in a pair); in tests with a fourth pair of males, three of four females agreed on the same male ($p > 0.05$). There was no relationship between our appraisal of male coloration and female preference, nor did females

prefer the larger of the two males when there was a size difference. Rather, male courtship effort may have influenced female choice. These experiments suggest that variance in lifetime male mating success can originate from congruent female preferences for certain males and rejection of others, and result in sexual selection through female choice in *Tropheus*.

THE ROLE OF NATURAL SELECTION IN SPECIATION – DATA FROM AFRICAN CICHLID FISHES

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The Great Lakes of East Africa with their species flocks of cichlid fishes provide excellent model systems for the study of adaptive radiation, an evolutionary phenomenon during which a multitude of species emerges within a short period of time. For the theory of adaptive radiation the understanding of the role of natural selection as driving force of diversification in natural populations is a critical issue. We study the role of adaptive evolution in two selected sets of model species, the first at an early stage of radiation in a satellite lake of Lake Victoria, and the second at a mature stage in Lake Tanganyika. Using a combination of comparative morphological, population genetic and quantitative genetic methods we addressed three major issues: (1) Do morphological differences among populations or species have an inherited basis? (2) Do the observed eco-morphological differences bear the signature of natural

selection? (3) What is the relative importance of divergent and stabilizing selection, enforced by competitive interactions, in evolutionary young and mature stages of adaptive radiation? Therefore, we reconstructed quantitative trait evolution in the wild, through joint analysis of relatedness and quantitative trait variation at the population level of traits critical for ecological specialization and assessed the presence and direction of selection by comparing neutral genetic variation of microsatellite markers to morphological variation of ecologically relevant quantitative traits. To demonstrate selection-driven evolution against the null-hypothesis of evolution by random genetic drift we use a combination of GST/QST comparisons by applying the “animal model approach” to natural populations.

PHYLOGENETIC ANALYSIS OF THE INTESTINAL MICROFLORA OF *THYMALLUS BAICALENSIS* DYBOWSKI, 1874

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Modern molecular analysis including 16S rDNA sequencing as well as fluorescence *in situ* hybridization become powerful instrument to solve variety of tasks on molecular ecology of microorganisms. Recently, this approach was successfully applied for intestinal microbiota investigations especially in fish and aquatic animals. It is routinely used for strain identification, analysis of uncultivable microorganisms as well as for developing PCR detection of fish pathogenic bacteria in practical usage.

The main aim of present research was phylogenetic diversity investigation of the intestinal microorganisms of *Thymallus baicalensis* from Lake Baikal with the adapted molecular methods.

Three specimens of *T. baicalensis* had been hatched at the south basin of Lake Baikal in May 2008, fish were killed by a blow to the head and fragments of mid and hindgut used in the experiments for DNA isolation. The obtained sequences were compared with previous data for *T. baicalensis* from Angara River, Irkutsk water reservoir and aquariums of Baikal Museum as well as with BLAST search in GenBank. We have shown that adapted methods allowed to retrieve variety of sequences belonged to phyla Proteobacteria and Tenericutes. Mainly facultative anaerobes (*Salmonella*, *Pseudomonas*, *Aeromonas*, *Mycoplasma*, *Escherichia*) were found among close relatives. Rare obligate aerobes (*Azospirillum*, *Caulobacter*, *Sphingomonas*, *Comamonas*, *Delftia*) characteristically showed the capacity to degrade complex organic compounds. It is essential that sequences of unculturable bacteria were obtained with the same approach from the intestinal microbiota of fish, termites, and variety of other organisms. Our special interest was devoted to Mycoplasma-like sequences those showed low homology (95%) with sequences of *Mycoplasma moatsii*, but maximum homology (98%) with sequences from the intestinal flora of Antarctic notothenioid fish. The Pseudomonas-like sequences were grouped into several groups which fallen down with another sequences of symbiotic microorganisms on phylogenetic trees.

So, we determined two groups of sequences Mycoplasma and Pseudomonas to be of interest for future molecular analysis and ecological research on the intestinal microorganisms of *T. baicalensis*. The work was supported by the Programme of Russian Academy of Sciences “Biological diversity” №44 (2008–2012).

A NOTE ON *SALMO LETNICA*, THE ENDEMIC OHRID TROUT, POPULATION STRUCTURING

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The Balkan Lake Ohrid contains a wide range of endemic species diversity considered to be caused by the long isolation of the lake from outside water systems, continuity of existence and exceptional geological age. Salmonid fishes constitute a noteworthy characteristic of Lake Ohrid biodiversity.

The relict nature and taxonomic value of the endemic, an endangered trout species *Salmo letnica* has been a disputed issue and is still controversial. According to various authors, this species in fact comprises three distinct populations, probably different intra-lacustrine forms inhabiting the lake: *Salmo letnica typicus*, *S. l. aestivalis* and *S. l. balcanicus*. These populations differ in their intralacustrine distribution, ecology and the spawning season. A further stock occurring in Lake Ohrid has been described: *Salmo letnica lumi*, a river form, inhabiting the tributaries of the western shore of the lake. However, the existence of genetic population structuring within Lake Ohrid has been questioned.

We provide an insight into genetic structuring of the *Salmo letnica* population inferred by variation at microsatellite loci. The data presented appear to support the notion of the population structure departure from its pristine state, due to erroneous management and stocking practices.

CHANGES IN DISTRIBUTION OF THE MACROPHYTES IN THE LAKE OHRID

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The macrophytic vegetation is quite distributed in the littoral zone of the Lake Ohrid and it includes emergent, floating and submerged plants. In the Lake there are the following belts of macrophytic vegetation: belt of *Cladophora*; belt of reed, *Phragmites*; belt of pondweed, *Potamogeton* and belt of stonewort, *Chara*.

In the following work the changes of the distribution of macrophytes from 11 localities of the littoral zone of the Lake Ohrid during a longer period of time are presented. The mentioned localities are: Radozda, Kalista, Struga, Grasnica, Caneo, Pristaniste, Mazija, St. Stephan, Pestani₁, Pestani₂ and St. Naum. In fact, a comparison has been made between our researches from the period 2006-2008, with the researches conducted by Jakovlevic in 1936.

The results indicate that there are changes in the horizontal as well as in the vertical distribution of the macrophytes if compared to those obtained in 1936.

The largest changes in the horizontal distribution have been evidenced in the locality of Struga (according to our researches 1535 meters, while according to Jakovlevic 860 meters). There are relatively large changes verified in the locality Pristaniste (our investigations yielded 648 meters while according to Jakovlevic – 340 meters), Grasnica (according to our

examinations 680 meters and according to Jakovlevic 440 meters), Mazija (in accordance with our researches 665 meters while according to Jakovlevic 470 meters), and St. Stephan (in accordance with our researches 380 meters while according to Jakovlevic 190 meters).

The largest changes in relation to the vertical distribution have been documented in the locality Grasnica (our studies yielded 14 meters while those from Jakovlevic 10,5 meters), Struga (in accordance to our researches 13 meters and according to Jakovlevic 11,4 meters), and Pristaniste (according to our researches 18,5 meters and according to Jakovlevic 17,6 meters).

According to our researches the largest changes in the horizontal as well as in the vertical distribution have been evidenced in the localities Grasnica, Struga and Pristaniste.

The changes of the horizontal and vertical distribution of the macrophytes are affected, above all, by the increased anthropogenic influence in the Lake Ohrid during the last seven decades.

COMPARATIVE ANALYSIS OF ICHTHYOFAUNA BETWEEN ANCIENT LAKES OHRID AND PRESIPA

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In Lake Ohrid, 20 native fish species are recognized: *Alburnoides ohridanus* (Karaman, 1928), *Alburnus scoranza* (Heckel et Kner, 1858), *Anguilla anguilla* (Linnaeus, 1758), *Barbatula sturanyi* (Steindachner, 1892), *Barbus rebeli* Köller, 1925, *Chondrostoma ohridanus* Karaman, 1924, *Cobitis ohridana* Karaman, 1928, *Cyprinus carpio* Linnaeus, 1758, *Gobio ohridanus*, Karaman, 1924, *Pachychilon pictum* (Heckel et Kner, 1858), *Pelagus minutus* Karaman, 1924, *Phoxinus limaireul* Schinz, 1840, *Rutilus ohridanus* (Karaman, 1924), *Salmo aphelios*, Kottelat, 1997, *Salmo balcanicus* Karaman, 1928, *Salmo letnica* Karaman, 1924, *Salmo lumi* Poljakov, Filip & Basho 1958, *Salmo ohridanus* Steindachner 1892, *Scardinius knezevici* (Bianco & Kottelat, 2005) and *Squalius squalus* Bonaparte, 1837.

In Lake Prespa, 11 native fish species are recognized: *Alburnoides prespensis* Karaman, 1924, *Alburnus belvica* Karaman, 1924, *Anguilla anguilla* (Linnaeus, 1758), *Barbus prespensis* Karaman, 1924, *Chondrostoma prespense* Karaman, 1924, *Cobitis meridionalis* Karaman, 1924, *Cyprinus carpio* Linnaeus, 1758, *Pelagus prespensis* Karaman, 1924, *Rutilus prespensis* Karaman, 1924, *Salmo peristericus* Karaman, 1938 and *Squalius prespensis* Fowler, 1977.

The autochthonous fish population of the Prespa Lake is rich with endemic species. Almost all cyprinid species (81, 81% of them) in the Prespa Lake, apart from the carp and the eel are endemic. Besides, in the Lake Prespa the exclusive autochthonous trout is also endemic. Unlike the Prespa Lake, in Lake Ohrid there are almost no endemic cyprinid species which would be characteristic only for this lake. The endemism of the cyprinid species from the Lake Ohrid is common for Ohrid-Drim-Skadar water ecosystem. Then again in the Lake Ohrid there is high endemism (100%) for all autochthonous salmonid species.

The large number of endemic cyprinid species of fishes in the Lake Prespa which are not attribute for the Lake Ohrid indicate to the old age of this lake and to the extensive independent development. The communication between these two lakes (which existed in the history) is not present any longer and the period from this termination is so long that the common cyprinid species managed to develop in entirely new species which are characteristic for the Ohrid and Prespa Lake independently. Despite the confirmed underground connection between the two lakes, the entirely different composition of the ichthyofauna indicates that the flow of living organisms or of fertilized roe through it was in the past period, and now it is impossible.

The similarity between the Ohrid cyprinid fauna with that of the other water ecosystems of the Ohrid-Drim-Skadar system point toward the conclusion that the development of this water system at least regarding the colonization of the cyprinid component is parallel in time and it is rather close concerning time. The absence of salmonid elements from the Lake Ohrid in the other parts of the Ohrid-Drim-Skadar system is due to, among other reasons, the different ecological circumstances present in the river Drim and the Lake Skadar.

THE CHECKLIST OF THE LAKE OHRID MONOGONONT ROTIFERS

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Rotifers comprise a modestly sized phylum of tiny, bilaterally symmetrical, eutelic metazoans, with rich and fascinating diversity. Phylum Rotifera is containing two major groups: the heterogonic Monogononta and the exclusively parthenogenetic Bdelloidea. Monogononta contains 1570 species, the majority of which are free-living fresh or inland water taxa. They are ubiquitous, occurring in almost all types of freshwater habitat and form a relatively diverse constituent of the fauna of stagnant freshwater ecosystems.

The analysis of the monogonont rotifers of the Lake Ohrid revealed the presence of 70 taxa, representing 26 genera and 18 families. Fifty-nine species occurred in the littoral zone and only 11 species in the pelagic. The highest number of species in the littoral habitats occurred due to the different life conditions in this zone, which allows greater habitat diversification.

According to genera the greatest species richness was observed for *Trichocerca* (11 species), *Lecane* (11 species) and *Cephalodella* (6 species). Some of these species are among the most common Rotifera, characteristic for vegetated areas.

Rotifers of Lake Ohrid created taxonomically rich composition due to a wide variety of habitats they can inhabit.

**THE MORPHOLOGICAL VARIABILITY OF *PALLASEA CANCELLUS*
(PALLAS, 1772) (CRUSTACEA: AMPHIPOD) FROM LAKE BAIKAL
AND THE ANGARA RIVER**

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The changes in population of Baikal amphipod *Pallasea cancellus* (Pallas) from five remote local populations is described by comparing 21 morphological features. The population changes of individuals of different sexes, which allows the geographical variability of sexual dimorphism to be detected is shown. The morphological differences between crustaceans inhabiting different parts of Lake Baikal and the Angara River were estimated more precisely by analyzing the principal components.

Lake Baikal is inhabited by rich and diverse amphipod fauna which includes >250 species. It is known that mature females and males differ in body size (*Pseudomicruropus*, *Macrohectopus*), the arming of gnathopods (*Micruropus*), and body (*Acanthogammarus*). Sexual dimorphism was described only in a small number of species (~30%) and in a limited number of morphological features. Practically no studies of intraspecific morphological variability of Baikal amphipods of different sexes have taken place, so this was the goal of this study. The task was to determine and describe the intra- and interpopulation variability of morphometric features in individuals of different sexes from four remote local populations of this species in Lake Baikal and the Angara River.

Specimens of *P. cancellus* for this study were collected from four parts of Lake Baikal (Kultuk, Bolshiye Koty, Boguchanskaya, Olkhon populations) and from the Angara River. Of each sample, 20-50 mature females and males were taken for analysis, totaling 440 spec. Twenty-one morphological features were measured according to: the total body length, the length of first, second, and third segments of the antenna I stem; the length of fourth and fifth segment of the antenna II stem; the length and width of the propodi of gnathopods of the first and second pairs; the length and width of the basipodits of pereopodi of the third, fourth, and fifth pairs; the length and width of the telson; the length of the uropodi of the third pair; and the length of the outer and inner branches of the uropodi of the third pair.

The values of morphometric parameters of males were higher than those of females in all studied local populations of *P. cancellus* (the differences were significant at $p < 0.001$). Using PCA to study the morphometric features of individuals of different sexes showed that the first five PC explain 95% of the variables' variability, with first two PC's being accounting to ~91%. The first PC (PC 1) is explains the most of the features' variability.

This component has a high correlation ($r = 0.67-0.99$) to the analyzed features in all populations. The second PC (PC 2) gives ~5.7% of the total dispersion. Individuals of each sex form separate groups in each given population in the PC 1 and PC 2 space. Sexual dimorphism was found in local populations of *P. cancellus* amphipod. Male and female Angara, Bolshiye Koty and Olkhon populations are separated by a significant hiatus in the spacing of the first two principal components. In addition, in individuals of the Kultuk and Boguchanskaya populations the sexual dimorphism is pronounced to a lesser degree here. Males are closer to females in terms of size in the position of two principal components.

Morphometric differences in males and females from various local populations indicate the presence of a geographical or group variability of dimorphism. Active processes of its evolutionary changes are taking place in Lake Baikal. The morphometric differences from samples of the Kultuk and Boguchanskaya settlement show evidence of a significant breach of panmixis between population groups, as well as the high degree of their isolation. The rather high degree of isolation between the populations means they can be considered geographical allopatric races of the species.

MODERN ENIGMAS OF ANCIENT LAKE BAIKAL

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Lake Baikal is considered to be one of the most investigated ancient lakes of the globe. Within the last 40 years the age estimations of the lake postulated by geologists evolved from several hundred thousands to the now commonly accepted 30–35 Mya, without any direct connections to any marine environment in the past. The animal biodiversity list of Baikal includes 2640 species, at least, 56% of them are endemic; average annual species number newly described is 10–15. All variety of the viewpoints on the genesis and origin of Baikal fauna (depending on age and ancestry) can be subdivided into 3 groups: ancient, purely freshwater ancestry (Berg), or ancient freshwater ancestry with insignificant influence of marine forms (Kozhov, Martinson, Starobogatov) [I]; ancient, with significant influence of marine forms (Wereschagin) [II]; rather modern (2–

3 Mya) mostly freshwater ancestry, speciation processes took place after global geological cataclysms in the scale of the whole lake (Dorogostaisky, Taliev, Lukin) [III]. Hypothesis II was the most popular among biologists before broad introduction of molecular-biological methods since ca. 1995 (due to enthusiasm of Prof. Grachev). Surprisingly, many species flocks, earlier considered to be ancient and relict, show rather modern dates of active branching and diversification (=speciation) (Comephoridae flock – several hundred thousand years b.p.); only 3 (Turbellaria Baikalarctiinae; part of Oligochaeta Lumbriculidae and Gammaroidea) of 9 groups are currently considered to be most ancient (data of Timoshkin, Kaigorodova and Ogarkov respectively). So Hypothesis III (relating the age) received substantial arguments yet despite a lack of clear geological argumentation. Even more striking facts have come to light within the last 3 years. Investigating psammophylous communities of Baikal (2006–2009) we found several animals, morphologically very close to those inhabiting similar environments in seas. For example, the first record of the representatives of typically marine suprafamily Schizorhynchoidea in freshwaters, or a Proseriate, morphologically resembling marine Otoplanidae (data of Timoshkin et al). Forameniferans were described 2 years ago from the mineralized spring, located ca. 120 km away from the North Baikal (data of Okuneva and Takhteev). Do all of these discoveries mean, that the almost forgotten hypothesis of Wereschagin (1940) should be again taken into serious consideration? Which origin hypotheses should be chosen as the most up-to-dated and proven? Probably, the synthetic theory of Baikal fauna origin remains open for further discussion.

Intense reconsideration of the endemic status of the most important, principal pelagic algae of Baikal (*Aulacoseira baicalensis*, *A. skvortzowii*, *Cyclotella minuta*, *Peridinium baicalense*, *Gymnodinium baicalense*, etc.)

has taken place during the last 3 years: they were found widely distributed in a dozen of high mountain lakes in the north of Irkutsk district, Zabaikalye and Buryatia (data of Bondarenko and Genkal). A certain amount of Bryozoa, Rotifera, Bivalvia, Polychaeta, Nematoda species (data of Kamaltynov, Pomazkova, Slugina, Sitnikova, Naumova, Matveev, Timoshkin), the distribution areas of which were previously restricted to Baikal, were also found in the northern lakes and rivers. What kind of the geological scenario fits these biogeographical discoveries? After our 2000–2009 investigations at the interdisciplinary test site Berezovy we can state, that the biogeochemical factor should be considered as one of the most important in the endemic speciation of Baikal and the most important reason for the unusual biodiversity and benthos productivity development (analogous reasoning can be applied for other ancient lakes of rift and karstic genesis, such as Tanganyika and Ohrid). We have shown, that benthonic organisms (microbes, algae, lichens, sponges and other invertebrates) demonstrate clear, comparatively high preferences to grow over some of the bottom rock types. Moreover, attached plants and animals can strongly increase the underwater rock aqualis processes, destroying them directly (sponges) or indirectly (for example, significant day and night changes of pH – lichens, macrophytes). It means, that some groups of hydrobionts may destroy the rocks during their life activities, receive necessary nutrients (very important source for this ultra fresh water ecosystem) and pass them through the food chains. Relatively high estimations of the benthos productivity and biomass, found by us in the stony littoral of Baikal should be considered as one piece of indirect evidence for this supposition. So, is Baikal really one of the most investigated ancient lakes?

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CHARACTERISTICS OF THE *DREISSENA PRESBENSIS* POPULATION IN LAKE OHRID, MACEDONIA

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Shell morphometric characteristics, depth distribution, abundance and biomass of *D. presbensis* populations in the Ohrid Lake were studied. Six sites (Pristanishte, Sateska, Kosnozglobna, Metropol, Peshtani, Trpejca) were sampled in two seasons July and October. The results showed that *D. presbensis* belt was distributed at depths from 27-30 m to about 10-5 m. In July, the species was most abundant at depths of about 20 m at different substrates: sand, clay – Pristanishte, Metropol, Peshtani, Trpejca; mud – Kosnozglobna. At some sites (Pristanishte, Kosnozglobna, Peshtani), *Chara* belt occurred at depths of about 10 m, where only juvenile mussels were found. Most abundant was the population at Trpejca (1600 ind./m²), followed by these at Pestani (1075 ind./m²) and Kosnozglobna (1000 ind./m²). The highest biomass was recorded at Pristanishte (3446.5 g/m²), followed by Kosnozglobna (3206 g/m²). In October again the highest abundance was recorded at Trpejca (1925 ind./m²), Pestani (2600 ind./m²) and Kosnozglobna (2075 ind./m²). The lowest values were recorded at Pristaniste which is a slightly mesotrophic site with muddy substrate. The highest biomass was

recorded at Trpejca (4297 g/m²). The mean shell lengths (SL) of *D. presbensis* recorded at different sites varied from 21.42 mm to 27.18 mm; mean shell heights (SH) from 12.45 mm to 15.95 mm; and mean width (SW) from 11.46 mm to 14.46 mm. The mean SH/SL ratio was 0.58; mean SW/SL ratio 0.53; and mean SW/SH 0.91. The results were discussed in comparison with seasonal data from previous years.

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COMPARATIVE STUDY OF GENUS *BARBUS* (PISCES, CYPRINIDAE) FROM LAKE OHRID AND LAKE PRESPA BY RAPD ANALYSIS

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During the times when the region around the Prespa and Ohrid Lakes were within the Roman Empire it was known as Desaretija. Hence, the Ohrid Lake and the Prespa Lake along with the Small Lake are known as the Desaret Lakes. When the Desaret Lake began to diminish it was divided into several smaller lakes, i.e. Prespa, Ohrid, Korcansko and Balishtansko Lake. They were connected and their waters continued to leak into the Adriatic Sea. These lakes had no connection to the great basin of the Aegean Lake. The fauna of the Ohrid-Prespa region belongs to the Mediterranean and central European fauna. The first one came to this region from the region of the Adriatic Sea through the rivers Devol,

Shkumba and Crn Drim while the second from the area of the central European fauna with which this region is immediately connected. The fish fauna of the Ohrid Lake is more diverse. The reason for this is that these lakes divided one from the other so early that the changes in the fauna of the Ohrid Lake could not be relocated to the Prespa Lake as well. Surface communication between these lakes never existed, hence their relation was conducted through the Korcansko Lake and it finished at the onset of the diminishing of the ancient neogenic Desaretsko Lake.

With the RAPD-PCR amplification with the primers C02, C07, C08, C11, C15 and C16 differences were detected between the populations of *Barbus peloponnesius* of the Ohrid Lake and *Barbus prespensis* of Lake Prespa. The statistical analysis demonstrated that intrapopulation similarities between these two populations with the primers CO2 is estimated at 0,465; at 0,222 with the primer C07; at 0,373 with the primer C08; at 0,363 with the primer C11; at 0,456 with the primer C15; while with the primer C16 is 0,2. The RAPD markers revealed intrapopulation variation and differentiation of the barbel from these two research items. From this data it can be concluded that the dispersion and divergence of these two barbel populations, with the adaptation of the local habitats and by a process of speciation, belong to two different species.

However, the question of the taxonomy of the species of barbel that inhabits the Ohrid Lake and the river Drim still remains unanswered. Also, the results of the electrophoretic analysis of the starch gel demonstrated some differences among the population of the barbel which live in the Ohrid and Skadar Lake.

**MOLECULAR ARCHIVES OF CLIMATIC HISTORY: EXPLORING
PATTERNS OF GENOMIC DIFFERENTIATION IN ENDEMIC SPECIES
RADIATIONS OF ANCIENT LAKES (MOLARCH)**

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Little is known about the evolutionary response of species to global climate change because time scales are too long to be directly studied. Recent phylogenetic studies on taxa inhabiting ancient lakes suggested that climate-induced environmental changes can be reconstructed by

analyzing genetic patterns within and among species. In fact such evolutionary responses seem to coincide with climate driven lake level-fluctuations and to be concentrated within narrow periods of time. Yet, it remains unclear to what extent such changes occur simultaneously across taxonomic groups and ecological guilds.

Our goal is to combine phylogenetic and paleoclimate data to test how patterns of evolutionary diversification agree with predictions of three groups of hypotheses (i) the 'turnover pulse' and 'paleo-ecological incumbency' hypotheses, predicting that speciation pulses across major taxa coincide with times of major lake level changes; (ii) the 'ecological locking' hypothesis, also predicts simultaneous speciation pulses but not necessarily at times of major lake level changes; (iii) the 'individual response' hypothesis, predicts no correlation of speciation pulses across taxa. These hypotheses will be tested using mtDNA sequences from selected endemic taxa (fish, crustaceans) of Lakes Baikal and Tanganyika, the two oldest lakes on Earth. Patterns of intraspecific diversity (reflecting dynamics of population size changes) and of the synchrony of interspecific divergences across taxa are used to investigate the evolutionary effects of lake level changes on different time scales, in taxa occurring in different habitats, within lakes and across continents. Combined with paleoclimatic information we will attempt to elucidate the effects of global climatic induced changes on the more general dynamics of diversification, loss of variation, adaptive radiations and speciation events.

STATE AND RESULTS OF PRELIMINARY STUDIES AND GOALS OF ICDP DEEP DRILLING AT LAKE OHRID: THE SCOPSCO INITIATIVE

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Lake Ohrid is a transboundary lake belonging to the republics of Macedonia and Albania with approximately two thirds of its surface area in Macedonia and about one third in Albania. With more than 210 endemic species described, the lake is a unique aquatic ecosystem and a hotspot of biodiversity. Its importance was emphasized, when the lake was declared a UNESCO World Heritage Site in 1979, and was included as a target area of the International Continental Scientific Drilling Program (ICDP) as early as 1993.

Though the lake is considered to be the oldest, continuously existing lake

in Europe, the age and the origin of Lake Ohrid are not yet fully determined. Age estimations vary between one and ten million years and are concentrated around two to five million years, and both marine and limnic origin is proposed. Extant sedimentary records from Lake Ohrid cover the last glacial/interglacial cycle and reveal that Lake Ohrid is a valuable archive of volcanic ash dispersal and climate change in the central northern Mediterranean region. These records, however, are too short to provide information about the age and origin of the lake and to clarify the mechanisms controlling the evolutionary development leading to the extraordinary high degree of endemism. Concurrent genetic breaks in several invertebrate groups indicate that major geological and/or environmental events must have shaped the evolutionary history of endemic faunal elements in Lake Ohrid.

High-resolution hydroacoustic profiles (INNOMAR SES-96 light and INNOMAR SES-2000 compact) taken between 2004 and 2008, and multichannel seismic (Mini-GI-Gun) studies in 2007 and 2008 effectively demonstrate the interplay between sedimentation and active tectonics and impressively prove the potential of Lake Ohrid for an ICDP drilling campaign. The maximal sediment thickness is c. 680 m in the central basin, where there are no nonconformities or erosional features. Thus the complete history of the lake is likely to be recorded.

Deep drilling in Lake Ohrid would help (i) to obtain more precise information about the age and origin of the lake, (ii) to unravel the seismotectonic history of the lake area including effects of major earthquakes and associated mass wasting events, (iii) to obtain a continuous record containing information on volcanic activities and climate changes in the central northern Mediterranean region, and (iv) to better understand the impact of major geological/environmental events on general evolutionary patterns and on the shaping of an extraordinary

degree of endemic biodiversity as a matter of global significance. For this purpose, five primary drill sites were selected based on the results obtained from sedimentological studies, tectonic mapping in the catchment and detailed seismic surveys conducted between 2004 and 2008. The GLAD800 will be used for the recovery of the up to ca. 680 m long sediment sequences.

STUDIES OF OOCYTE GROWTH DYNAMICS IN TWO FORMS OF BAIKAL GRAYLING *THYMALLUS BAICALENSIS* (THYMALLIDAE)

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Lake Baikal is inhabited by several forms of grayling which have been regarded either as subspecies of Siberian grayling *Thymallus arcticus* (Pall.) or independent species. According to the latest data on molecular-genetic and morphological analyses, grayling *Thymallus baicalensis* Dyb. representing two ecological forms (white and black grayling) inhabit Lake Baikal. They differ in linear-weight parameters, age of sexual maturity, fat content, fecundity, habitat, conditions, and terms of reproduction.

Information on oogenesis of graylings has been very limited to date, and oogenesis is one of the main steps in the life cycle of organisms. On the whole, a large amount of research has been devoted to studies on peculiarities of fish oogenesis based on physiological, biochemical and morphological characteristics. Few specialist studies have been carried out on comparative analysis of oogenesis in different forms within one

species. Therefore, it is necessary to carry out comparative studies on size characteristics of oocytes in two forms of Baikal grayling *T. baicalensis* at the stage of the latest previtellogenesis, as well as at the beginning and in the middle of the phase of cytoplasm vacuolization.

The authors analyzed samples of immature and mature females of black grayling obtained from Bolshoy Ushkany Island (July 2003, August 2006) and only mature females from Cape Orlovsky (June 2003) and Khaga-Yaman Bay (June 2003). Samples of white grayling collected at Bolshoy Ushkany island (August 2006) consisted of mature individuals, while those from the Selenga shallow waters (July 2003) consisted of immature and mature individuals.

Size characteristics of oocytes were estimated with histological analysis. According to the data obtained, there were no size differences between oocytes of black and white graylings during the corresponding phases of development (at the phase of the latest previtellogenesis, at the beginning and in the middle of the phase of cytoplasm vacuolization). Paying attention the fact that oocytes of white grayling are smaller than oocytes of Black grayling at the phase termination of cytoplasm vacuolization and at the beginning of the phase of yolk deposition, it is possible to say that the known difference between the diameter of mature fish eggs of two forms of Baikal grayling is the result of the different speed of gaining trophic substances in oocytes of studied fishes and is defined by difference in oocyte development at the stage of forming yolk inclusion.

PRELIMINARY INVESTIGATIONS OF ODONATA FROM LAKE OHRID (MACEDONIA)

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There is much information about odonates in lakes in whole Europe. The lack of data about this group of invertebrates in Lake Ohrid - the UNESCO natural and cultural heritage - is unusual. The aim of our paper is to cover that field. Material included 17 species and 476 specimens of only adult Odonata, which was collected in June 2009 year on 21 stations of the Lake Ohrid. Such number of species is not very high and appears in low differential of phyto-littoral of the lake. The most numerous species is *Enallagma cyathigerum*, which is widespread and associated with nymphaeid and elodeid plant species. Such a high number of individuals of this species is supported by a large belt of elodeids (*Potamogeton perfoliatus*) occurring in Lake Ohrid. Four odonates belong to South-European species; the other ones are widespread European species. Probably the reason is the position of Lake Ohrid in the mountains, which made climate more moderate.

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