SEH 2015
18TH EUROPEAN CONGRESS
OF HERPETOLOGY

PROGRAMME AND ABSTRACTS

University of Wrocław, Poland
7-12 September 2015
Programme & Abstracts
XVIII European Congress of Herpetology,
7-12 September 2015, Wroclaw, Poland

Edited by Borczyk B., Ogielska M., Kolenda K., Skawiński T.
Organizing Institutions:
University of Wroclaw, Societas Europaea Herpetologica

Supporting Institutions:
Botanical Garden, Zoological Garden, Natural History Museum

Local organizing committee:
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Prof. Jacek M. Szymura (Poland)
Prof. Matthias Stöck (Germany)

Conference logo: Leszek Cielma
Book cover & cover photos: Anna Najbar
Dear Colleagues,

Welcome to Wrocław!
The SEH Congress is organized in different European countries; now for the first time in Poland. The 18\textsuperscript{th} SEH European Congress of Herpetology is hosted by the University of Wrocław and supported by the Wrocław ZOO.
Scientific presentations include 5 plenary lectures on topics of general interest by invited speakers and about 80 talks in parallel sessions. We have grouped them according to topics (evolution, phylogeny, biogeography, conservation, morphology, diseases and threats, physiology, reproduction, ecology, and conservation), doing our best in joining merit and time suitable for participants. The scientific program also includes more than 90 poster presentations; after the poster session we invite all participants for discussion supported by the Polish cider. Parallel to the sessions, a workshop entitled “Toward regional IUCN amphibian red list assessment” will be held.
Besides scientific presentations, some social events will take place including the opening ceremony, welcome get-together, and gala dinner.
We wish you a nice visit to our country and an interesting scientific meeting.

On behalf of the Local Organizing Committee

Bartosz Borczyk and Maria Ogielska
In Memoriam Professor Leszek Berger

The SEH 18th European Congress of Herpetology is dedicated to the memory of Professor Leszek Berger, Polish herpetologist and one of the major scientists of the Polish Academy of Sciences.

Leszek Berger was born 10 February 1925 in Pabianice (Łódzkie province). He spent his childhood and youth in Lewkowiec and Ostrów Wielkopolski (southern Wielkopolska region). Inspired by the richness of the local wildlife, he decided to study biology (1947-1950) at the Mathematics and Natural Science Faculty of the Poznań University. As a fourth-year student he began working as an assistant in the Natural History Museum in Poznań. His master’s and doctoral dissertations concerned the mollusks; however his further scientific work was devoted to amphibians. In 1963 he published a pioneer study on water frogs based on experimental crosses between different morphological forms of water frogs. He discovered that the edible frog *Rana esculenta* (now *Pelophylax esculentus*) was not a species but an interspecies hybrid between the pool frog *R. lessonae* (*P. lessonae*) and the marsh frog *R. ridibunda* (*P. ridibundus*). Further studies showed that the edible frog arose through backcrosses with one of its parental species and maintained its population thanks to hybridogenesis, a unique reproductive system. The gametes of hybrids contain only one of the genomes of the parental species while the other one is rejected from the germline prior to meiosis. In 1973 he received the first-degree award from the Polish Academy of Sciences for the discovery of a new type of heredity and reproduction in one of the most common amphibian species. On the basis of Berger’s results, zoologists around the world have begun to study water frogs, which resulted in discovery of many new water frog species. One of them, the former *Rana bergeri* Günther, 1985 (now *Pelophylax bergeri*), was named in his honor.

Over the course of 39 years (1963-2001) Professor Berger bred and reproduced 16 taxa of western Palearctic water frogs. He obtained over 800,000 offspring from approximately 1,500 crosses, being perhaps the most prolific scientific breeder of amphibians in the world. His scientific output includes over 120 publications.

Professor Leszek Berger passed away on 8 July 2012 at the age of 87. His resting place is in a cemetery near the Żurawiniec reserve in Poznań, where he began his research on amphibians.
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<td>Evolution revolution? On the origins and maintenance of venom in snakes</td>
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<td>ANITA MALHOTRA</td>
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<td></td>
<td>School of Biological Sciences, Bangor University, Gwynedd, UK</td>
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<td>9.30-10.50</td>
<td>Evolution and phylogeny</td>
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<td>9.50-10.10</td>
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<td>10.30-10.50</td>
<td>Pabijan M., Zieliński P., Dudek K., Stuglik M., Babik W.: A multilocus dataset for <em>Lissotriton</em> newts delimits taxa but fails to recover evolutionary relationships</td>
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<td>Hallerman J., Schmitz A.: The <em>Boaedon fuliginosus</em> complex – phylogenetic analyses</td>
<td>Toward regional IUCN amphibian red list assessment Session 1. IUCN Red List</td>
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<td>11.40-12.00</td>
<td>Hartmann T., Ihlow F., Flecks M., Schmitz A., Rödder D.:</td>
<td>Disentangling the Calotes mystaceus complex</td>
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<td>Zinenko O., Sovic M., Joger U., Gibbs L.:</td>
<td>Analysis of phylogeny and introgression in small Eurasian vipers using RADseq data</td>
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<td>14.30-15.30</td>
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<td>PIERRE JOLY</td>
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<td>UMR5023, Laboratory of Ecology of Natural and Man-impacted Hydrosystems</td>
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<td>9.09.2015 Wednesday</td>
<td>Plenary lecture</td>
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<td>Differences in the ecomorphological relationships between mainland and island Anolis lizards</td>
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<td>ANTHONY HERREL(^1) &amp; JONATHAN LOSOS(^2)</td>
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<td>1UMR 7179 C.N.R.S/M.N.H.N., Département d’Ecologie et de Gestion de la Biodiversité, 57 rue Cuvier, Case postale 55, 75231, Paris Cedex 5, France.</td>
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<td>2Harvard University, Museum of Comparative Zoology, 26 Oxford Street, Cambridge, MA 02138, U.S.A.</td>
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<td>Room 1</td>
<td>Phylogeny and taxonomy</td>
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<tr>
<td>9.30-10.50</td>
<td>Zieliński P., Nadachowska-Brzyska K., Dudek K., Babik W.:</td>
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<td>Divergence history of the Carpathian and smooth newts</td>
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<td>Session 2. Threatened amphibian species in Europe – are there something new?</td>
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<td>10.10-10.30</td>
<td>Padhye A., Modak N., Dahanukar N.: Preliminary molecular clock analysis of Western Ghats endemic genus <em>Indirana</em></td>
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<td>11.40-12.00</td>
<td>Velo-Antón G., Buckley D., Martínez-Solano I., Canestrelli D., Lourenço A., Martínez-Freiría F.: Reconstructing the biogeographic history of geographically disjunct populations: the case of <em>Salamandra s. bernardezi</em> and <em>S. s. giglioli</em></td>
<td>Toward regional IUCN amphibian red list assessment Session 2. Threatened amphibian species in Europe – are there something new? Amphibian Red List Assessors Team for European Region &amp; I Conservation Task Force Moderators: Jelka Crnobrnja-Isailović &amp; Raoul Manenti</td>
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<tr>
<td>Time</td>
<td>Conservation</td>
<td>Habitats – amphibians</td>
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<td>12.20-12.40</td>
<td>Ghielmi S., Menegon M., Marsden S.J., Laddaga L., Erne B., Ursenbacher S.: Genetic diversity within the adder (<em>Vipera berus</em>) in Italy and surrounding regions</td>
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<td>14.30-15.50</td>
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<td>15.30-15.50</td>
<td>De Vries W., Marco A., van de Loo M.: EPMAC: A reference tool for the evaluation of regional research on amphibians</td>
<td>Sinsch U.: Do displaced newts (<em>Triturus cristatus, Lissotriton vulgaris</em>) navigate?</td>
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<tr>
<td>17.00-17.20</td>
<td>Lourenço A., Álvarez D., Velo-Antón G.: Trapped within the city: the effects of recent isolation on population structure and demography of <em>Salamandra salamandra</em> in Oviedo</td>
<td>Ćorović J., Popović M., Cogălniceanu D., Carretero M.A., Crnobrnja-Isailović J.: Species distribution modelling of <em>Darevskia praticola</em> from the Balkan Peninsula</td>
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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>18:00</td>
<td>SEH Council meeting</td>
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</tbody>
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**10.09.2015 Thursday**

**8.30-9.30**

**Plenary lecture**

Examining the ecophysiology of Mediterranean lizards with evolutionary, biogeographic and conservation eyes

**MIGUEL A. CARRETERO**

*CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, N° 7. 4485-661 Vairão, Vila do Conde, Portugal*

**9.30-10.50**

**Morphology and evolution**

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<tr>
<td>9.50-10.10</td>
<td>Erens J., Miralles A., Vences M.: The evolutionary relationships of Malagasy scincines – a potential future model clade to study limb</td>
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**Room 2**

**Workshop**

Toward regional IUCN amphibian red list assessment

**Session 3. Proposed regional Red List status for newly described amphibian species in Europe**
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<tr>
<td>10.10-10.30</td>
<td>Evolution in squamate reptiles</td>
<td>Reinhard S., Kupfer A.: The evolution of sexual dimorphism in salamandrids: patterns and processes</td>
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<tr>
<td>10.30-10.50</td>
<td>Study of embryonic development of the lemon-yellow tree frog, <em>Hyla savigni</em> Audouin, 1827 in comparison with common tree frog <em>Hyla arborea</em> (Linnaeus, 1758)</td>
<td>Karanlık S., Najafi-Majd E., Yildirim E., Kaya U.:</td>
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<tr>
<td>10.50-11.20</td>
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<td>11.20-12.40</td>
<td>Morphology</td>
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<td>11.20-11.40</td>
<td>Computer vision-based method for recognition of individual patterns in amphibians</td>
<td>Eckhardt F.S., Döring K.W.:</td>
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<td>11.40-12.00</td>
<td>Comparative skeletal morphology of four species of the genus <em>Neurergus</em> (Caudata: Salamandridae)</td>
<td>Najafi-Majd E., Yildirim E., Kaya U., Safaei-Mahroo B., Kumlu taş Y., Ilgaz Ç.:</td>
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<tr>
<td>12.00-12.20</td>
<td>Comparative skeletal osteology in three scincid lizards (genus: <em>Ablepharus</em>) from Turkey</td>
<td>Yildirim E., Kumlu taş Y., Ilgaz Ç.:</td>
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<td><em>Cretaceous World</em> – A 99 million-year-old lizard fauna in Burmese amber fills evolutionary gaps</td>
<td>Wagner P.:</td>
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<td>Introduced species threats</td>
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<tr>
<td>14.50-15.10</td>
<td>Denoël M., Winandy L.</td>
<td>Consequences of fish introduction and extirpation on populations of metamorphic and paedomorphic newts</td>
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<td>Escoriza D., Ben Hassine J.</td>
<td>Niche partitioning at local and regional scale in the North African Salamandridae</td>
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<td>15.10-15.30</td>
<td>Winandy L., Darnet E., Denoël M.</td>
<td>Newts skip aquatic life and forego reproduction in response to alien fish introduction</td>
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<td>Ficetola G.F., Lunghi E., Canedoli C., Padoa-Schioppa E., Pennati R., Manenti R.</td>
<td>Niche evolution in European <em>Hydromantes</em>: mismatches between macroecological and fine-scale analyses</td>
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<td>15.30-15.50</td>
<td>Spikmans F., Ouborg J.</td>
<td>Genetics of Dutch Wall Lizards (<em>Podarcis muralis</em>); on the vitality of the only native population and the threat of origin of introduced populations</td>
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<td>Žagar A., Vrežec A., Carretero M.A.</td>
<td>Do they compete or not? Using a combination of approaches to understand the competition between two similar lizard species</td>
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<td>15.50-16.20</td>
<td>Coffee break</td>
<td>Coffee break</td>
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<tr>
<td>16.20-17.40</td>
<td>Diseases and threats</td>
<td>Climate</td>
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<td>16.20-16.40</td>
<td>Spitzen A., Bogaerts S., Woeltjes T., Pasmans F., Martel A.</td>
<td>Is the amphibian pet trade a vector for <em>Batrachochytrium salamandrivorans</em> in the Netherlands?</td>
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<td>Veith M., Kieren S., Göçmen B., Sotiropoulos K., Lötters S.</td>
<td>How to survive past climate changes – lessons from <em>Lyciasalamandra</em></td>
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<td>16.40-17.00</td>
<td>Spitzen-van der Sluijs A., Bosman W., Spikmans F., Pasmans F., Martel A.</td>
<td>The uncertain future of the Dutch fire salamander population infected with <em>Batrachochytrium salamandrivorans</em></td>
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<td>Salvidio S., Oneto F., Ottonello D., Pastorino M.V.</td>
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<td>Sinsch U.</td>
<td>Winter survival of juvenile toads (<em>Epidalea calamita, Bufotes viridis</em>) is the principal driver of population dynamics</td>
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<td>8.30-9.30</td>
<td><strong>Lecture</strong></td>
<td>WIESŁAW BABIK</td>
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<td><em>Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland</em></td>
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<td>11.20-12.40</td>
<td>Physiology</td>
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<td>Loulida S., Naimi M., Znari M.,</td>
<td>Tolerance to salinity</td>
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<td>Bendami S., Moumane A., Maran</td>
<td>and dehydration in</td>
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<td>J.: Tolerance to salinity and</td>
<td>the Sahara Desert’s</td>
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<td>dehydration in the Sahara</td>
<td>Blue-eyed Turtle,</td>
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<td>Desert’s Blue-eyed Turtle,</td>
<td><em>Mauremys</em> <em>leprosa</em></td>
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<td><em>leprosa</em> <em>saharica</em> <em>Firtz,</em></td>
<td><em>saharica</em> <em>Firtz,</em></td>
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<td>2006 (Testudines: Geoemydidae)</td>
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<td>Southern Morocco</td>
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<td>Bendami S., Loulida S., Naimi M.,</td>
<td>Effects of</td>
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<td>Znari M., Moumane A.: Effects</td>
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<td>Colonization by <em>Salamandra infraimmaculata</em> of new breeding sites in xeric habitats</td>
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<td>Creemers R.C.M., Joosten K., van Grunsven R.</td>
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<td>Ilić M., Stamenković G., Nikolić V., Marković V., Marinković N., Paunović M., Crnobrnja-Isailović J.</td>
<td>Identification of syntopic anuran species at early tadpole stages: could morphometric analysis resemble molecular genetics?</td>
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<td>Halpern B., Sós E., Walzer C., Péchy T.</td>
<td>Post-release survival of Hungarian meadow vipers (<em>Vipera ursinii rakosiensis</em>)</td>
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<td>Melotto A., Ficetola G.F., Denoël M., Manenti R.</td>
<td>Predator cues and risky habitats affect foraging activity in salamanders</td>
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<td>17.40-18.00</td>
<td>Krása A.</td>
<td>Amphibian road mortality mitigation in the Czech Republic</td>
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**Coffee break**

**Ecology and conservation**

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**Closing ceremony**

**12.09.2015 Saturday**

**Departure for the trip**
Plenary lectures
Divergence and gene flow in *Lissotriton* newts

*Wiesław Babik*

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Speciation is often a prolonged process and in consequence taxa at various stages of divergence occur in nature. Reconstructing relationships between such recently diverged and incompletely reproductively isolated lineages is challenging. But such systems also provide excellent opportunities to study poorly understood historical patterns and consequences of genetic exchange between diverging species. I will discuss our work on divergence and gene flow in newts from the *Lissotriton vulgaris/L.montandoni* group. Several distinct but closely related evolutionary lineages are present in this group. We used various classes of molecular markers to obtain information about the relationships, extent and historical patterns of gene flow among these lineages. Transcriptome sequencing has been instrumental in obtaining genome-wide polymorphism data in this system and I will argue that it is the methodology of choice in other species with large complex genomes, such as many amphibians. We found that both contemporary and historical gene flow between several more distantly related lineages appear stronger than between more closely related lineages. Gene flow has been heterogeneous across the genome with some genes, for example those of the Major Histocompatibility Complex (MHC), introgressing more easily than others. The *Lissotriton* system is particularly well suited to test the hypothesis about preferential introgression of genes evolving under balancing selection. This is because patterns of gene flow in this group facilitate distinguishing ancestral polymorphism, particularly common in genes evolving under balancing selection, from introgression. I will outline the approach we use to test this hypothesis and present the results obtained so far in this ongoing project.

**Key words:** *Lissotriton*, hybridization, adaptive introgression, balancing selection, transcriptome
Evolution revolution? On the origins and maintenance of venom in snakes

ANITA MALHOTRA

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Recent application of technological advances to the study of snake venom has overturned many of the conventional ideas about how snakes evolved their venom in the first place, and what drives the continuing evolution and maintenance of the diversity of toxins that they possess. However, there are a number of gaps in our understanding that require breadth (increased taxonomic coverage), as well as depth (increased genomic coverage), before we gain a real insight into this process.

Key words: venom, next-generation sequencing, genomics, transcriptomics, phylogeny, recruitment
Examining the ecophysiology of Mediterranean lizards with evolutionary, biogeographic and conservation eyes

MIGUEL A. CARRETERO

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Coping with environmental variations is a major challenge for organisms, particularly those that are ectotherms and poor dispersers. Temperate lizards provide a nice ecophysiological model since they are abundant and easy to manipulate while in nature they have to face daily and annual cycles in temperature and humidity. The way they respond to such changes has profound consequences on their current distribution at different spatial scales as well as on the interactions between species with different ecophysiological traits. Also, the way ecophysiological traits respond to long term environmental trends along the evolutionary axis (either by staying conservative or shifting) has likely contributed to range expansion/restriction, lineage diversification and lineage extinction. Finally, both current and historical ecophysiology has repercussions on the species conservation status and management strategies, especially if scenarios of climate change for the next century are confirmed. I will illustrate all these aspects using recent investigations on thermal and hydric physiology of the lizards from the Mediterranean Basin based on a combination of field observations, lab experiments and ecological models on an explicit phylogenetic and paleoecological framework. Directions for future research in this area are suggested.

Key words: preferred temperatures, water loss rates, fundamental niche, mechanistic models, climate change, Lacertidae
Differences in the ecomorphological relationships between mainland and island *Anolis* lizards

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²Harvard University, Museum of Comparative Zoology, 26 Oxford Street, Cambridge, MA 02138, U.S.A.

Over the past decades, *Anolis* lizards have become a model for the study of convergence and adaptive radiations due to the manyfold radiation of species with similar morphologies in ecological similar niches. However, *Anolis* lizards have radiated extensively on the mainland of Central and South America with over half of the 400+ known species being found in continental settings. Recent analyses of ecomorphological relationships have shown that mainland and island species differ considerably. For example, for a given limb length Caribbean anoles run faster than their mainland counterparts. Conversely, for a given head dimension mainland anoles bite harder than Caribbean anoles do. These differences are thought to be due to differences in predation pressure on islands versus mainland ecosystems. Here we present data on muscle anatomy, performance, ecology, and behavior for over 50 species of *Anolis* and demonstrate that differences in performance are not due to differences in muscle properties, but rather in the size and shape of the skeletal elements. Caribbean anoles are however different from mainland anoles in their behavior, especially in males. Not only do male Caribbean anoles display more than do mainland anoles, they also move more. Moreover, sexual dimorphism in overall movement patterns observed in Caribbean anoles is not observed on the mainland where both sexes move little. Finally, significant differences in escape behavior are also observed between mainland and Caribbean anoles with mainland anoles relying more on crypsis than on flight. These results are in accordance with the idea of a higher predation pressure in mainland ecosystems and may explain the striking divergence in ecomorphological relationships between both radiations.

Key words: lizard, evolution, performance, diversification, ecomorphology
Life histories and conservation in amphibians

PIERRE JOLY

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The distribution of most endangered amphibians is strongly constrained by both the presence of predatory fishes in breeding sites and the fluctuation of water levels that have shaped specific adaptations and life histories. Beside adaptations of breeding behaviour and larval development, my aim is here to focus on the interplay between time and space at the scale of the whole lifetime.
For a long time, the theory of life history evolution has pointed out longevity as a response to fluctuations of reproductive success. A long life span makes it possible to spread the risk of reproductive failure over several reproduction opportunities (iteroparity). Long life span and iteroparity are observed in many amphibians, particularly in small-bodied species for which a short life span is expected. A long life span supposes to be well protected against predators and to avoid the detrimental effects of oxidative stress (slow aging strategy). However, reducing metabolism could negatively impact the investment in future reproduction. Long-term studies reveal that females of iteroporous amphibians often skip reproduction, thus reducing the positive effect of iteroparity on reproductive success in fluctuating environments.
Dispersal is another way of spreading the risk of reproduction failure over space. Searching at each breeding occasion a high quality site is an option that has for a long time been considered as costly. However, both CMR data and estimation of genetic distances have revealed that nomadism, i.e. changing breeding site at each breeding event, is frequent, even in small-bodied species.
The conservation of many amphibians thus addresses three main questions:
- how to manage fluctuating water conditions at breeding sites?
- how to manage landscape for allowing dispersal?
- how to deal with global warming when slow species are at risk of being accelerated by higher temperatures?

Key words: conservation, life histories, iteroparity, dispersal, amphibians
Oral presentations
Diversification of the skinks of the *Mabuya* group: novel relationships and historical biogeography

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With approximately 1600 extant species, skinks represent a major component of squamate diversity and have a nearly cosmopolitan distribution. The *Mabuya* clade (recognized by some as the Mabuyidae) sensu lato which comprises more than 200 species distributed in tropical Asia, the Middle East, Africa, South America and several islands and island groups in the Indian and Atlantic Oceans. Depending upon the taxonomy adopted, the group consists of between six and 20 genera and has had a long history of systematic controversy. In order to investigate the higher-level relationships and diversification across their range, we assembled a dataset that included representatives from all genera and main clades of this group, with a concentration in the Old World lineages. We generated sequences for 11 genes and a total of more than 8400 bp, and performed concatenated and species tree analyses. The robust results obtained reveal formerly unrecognized groups and novel relationships, and point to an Asian origin for the diversification of this clade. We investigated the timeframe of this diversification through the use of alternative dating strategies and reconstructed possible biogeographic scenarios. Alternative calibration schemes yield differing results, but major cladogeneic events are likely to have occurred between the Late Eocene and Mid Miocene, implicating overwater dispersal for most intercontinental and insular movements. Overall, employing comprehensive taxonomic and increased molecular sampling, we were able to confidently reconstruct the phylogenetic relationships and historical biogeography of this skink clade that has greatly diversified across three continents and numerous insular complexes.

**Key words:** Scincidae, species tree, biogeography, molecular phylogenetics, taxonomy
Altitudinal variation in three populations of Darwin’s frogs
(Rhinoderma darwinii)

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Altitude can create environmental variation in temperature and seasons length that may influence life histories of ectothermic organisms. Additionally, in polymorphic amphibian species, the interaction of habitat characteristics (temperature and light) and genotype determines the expression of colour and patterns, and thereby mating behaviour, reproduction, predation and morph ratio.

In the present study, we analyzed the effect of altitude on microhabitat and Darwin’s frogs (Rhinoderma darwinii) characteristics at three sites near Coñaripe (South of Chile): Villarrica National Park (Villarrica NP; 1100 m asl), Vergara Hot Springs (Vergara HS; 800 m asl) and Parque de las Aguas (PA; 400 m asl). Results showed that population characteristics and microhabitats differed among study sites. Population structure was similar at lower altitude populations (Vergara HS and PA), but individuals were more abundant at middle elevation site (Vergara HS). Microhabitat characteristics like temperature, vegetation ground cover, canopy openness, number of vegetation strata and background substrate colour differed among sites. Frogs at lower altitude sites (Vergara HS and PA) were more frequently found in microhabitats with warmer temperatures, open canopy and more variable background substrate colour. Individuals showed significant differences among sites in corporal measurements (SVL and TL), body colours and dorsal pattern frequencies. Individuals at the lowest altitude site (PA) were significant smaller than those at higher altitude sites (Villarrica NP and Vergara HS). Individuals at lower altitudes (PA and Vergara HS) exhibited a larger variability of body colours and dorsal patterns than those of the high altitude population. Our findings showed a great variability of Darwin’s frog characteristics associated with altitude. The influence of microhabitat on individual and population variability is highlighted, but further studies are needed to fully understand the underlying mechanisms maintaining frogs’ variability. Such insights are needed to preserve the evolutive potential of Darwin’s frogs and with that long-term population viability of the species.

Key words: altitude, individual and population characteristics, microhabitat, anuran
When do we need more data before making conservation decisions? 
An example from a turtle reintroduction

Stefano Canessa¹,²,⁷, Gurutzeta Guillera-Arroita², José Lahoz-Monfort², Darren M. Southwell², Doug P. Armstrong³, Iadine Chadès⁴, Robert C. Lacy⁵, Sarah J. Converse⁶

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Decisions about the conservation of endangered species are usually made in the face of uncertainty, given our limited knowledge of species and systems and their inherent variability. As a result, we often advocate further research, with the assumption that after obtaining more information we will be able to make a better decision. On the other hand, we cannot learn everything there is to know, and the resources and time to learn are often limited.

In the northern Italian region of Liguria, a recovery plan for the European pond turtle *Emys orbicularis* has been in place since the late 1990s, with a captive breeding program producing individuals for reintroduction. With a full-scale reintroduction plan to be implemented in the years 2014-2016, a decision must be made about the age at which individuals should be released, in the face of uncertain survival rates. A trial reintroduction might help us solve some of that uncertainty, but will it be enough to improve the long-term results of the reintroduction?

We use an analytical tool called value of information analysis to evaluate the benefit of running a trial reintroduction. We show that a trial release of 3-yr-old turtles will prove more informative than a release of 5-yr-olds. The information we might collect from the trial should allow us to increase the survival of juvenile turtles by up to 6%.

When we think we need additional information before making a conservation decision, we need to consider three things: (1) what do we already know? (2) how good is the information that we can collect? (3) how will it change our decision? The use of analytical tools to consider these aspects quantitatively allows more rigorous and transparent decisions. Moreover, we can allocate resources more efficiently, to focus on solving those uncertainties that really make a difference.

**Key words:** Captive breeding; conservation; decision-making; management; survival; translocations
Species distribution modelling of *Darevskia praticola* from the Balkan Peninsula

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The meadow lizard, *Darevskia praticola*, is listed as Near Threatened (NT) on the IUCN Red List but expanded habitat loss makes this species close to qualifying as Vulnerable (VU). The most noticeable threat comes from historical and contemporary cutting of oak forests that significantly reduced the suitable forest habitats throughout much of its range. Here we summarized the published geographic records of the meadow lizard’s occurrence in Europe with an emphasis on the westernmost part of its range (Serbia) and modelled the potential distribution on the Balkan Peninsula to get an insight into possibly overlooked habitats. Distribution of *D. praticola* was modelled with Maxent using precise occurrence records from Serbia and Romania and a variety of GIS layers rescaled to a resolution of 100 m²: BioClim, NDVI, Land Cover, slope and slope aspect. Analysis of variable contributions showed that Land Cover has the strongest effect on the model, followed by slope, climatic variables and NDVI vegetation indices. The results of a jack-knife analysis were similar, recovering Land Cover as the most important variable, followed by NDVI, slope and climatic variables. Regarding potential new localities, this model suggests existence of additional suitable habitats in central, eastern and southern parts of Serbia (including area of Kosovo), south-western Romania, across the Balkan Mountains in Central Bulgaria and also in the south of the country connected to the potentially suitable habitats in northern parts of Greece and Turkey. Such localities should be surveyed in the future to corroborate the predictive power of the model. The method provides a useful tool for directing fieldwork to areas with a higher probability of finding previously unknown populations.

**Key words:** Maxent, species distribution model
Effects of artificial light on amphibians (*Bufo bufo* and *Triturus cristatus*)

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Especially during spring migration many amphibians are killed by traffic when they cross roads moving to reproduction sites. Roads are often equipped with street lighting. Many nocturnal animals, including amphibians, are attracted by these lights; therefore street lighting may increase the impact of traffic on migrating amphibians. Until now it was unknown how this lighting affected amphibians and their seasonal migration patterns. Using experimental illumination (green, red, white light and a dark control) we tested whether light affected the migration under field conditions. The largest part of the field work was conducted in cooperation with volunteers, organised in local toad patrol groups. These were trained in a citizen science project to work according to protocols and collecting high quality field data. Common toads (*Bufo bufo*) avoided sections of roads that were illuminated with white or green light but not red light. By switching of the light for the second part of the night we showed that toads interrupt their movement when they come across illumination. Effects of artificial light on toads and on other species (e.g. *Triturus cristatus*) and in different seasons will be compared and discussed. Light with low levels of short wavelength can be used to mitigate effects of artificial light during spring migration.

**Key words:** amphibian, citizen science, light pollution, phototaxis, mitigation, conservation
Reevaluating IUCN Red List assessment on European Amphibians

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The last Red List assessment of European amphibians was completed and published in 2009. Ninety species were evaluated: one of them with status Data Deficient (DD), and another 21 listed as threatened on the basis of criteria A (population restriction), B (restricted geographic range), or D2 (very small or restricted population). The 54 other species were listed as Least Concern (LC) due to their large geographic ranges and lack of studies explicitly proving negative impacts of threats. The remaining 14 species were catalogued as Near Threatened (NT). Since 2009, the IUCN’s definition of the European region has changed, with Russia, Belarus and Ukraine included in the Northwestern Asia region, and Cyprus and Turkey in the Western Asia region. Following this new regional delimitation and updated taxonomic checklists used by the global amphibian IUCN Red List, only four amphibian species recognized after the last assessment can be included into the new IUCN regional Red List for Europe. These are: Triturus ivanbureschi, T. macedonicus, Bufo spinosus and Hyla molleri, and their Red List status is
currently Not Evaluated (NE). As the deadline for the next amphibian assessment round is getting close, changes in the regional conservation status of European species should be done where necessary. Apart from briefly analyzing the available evidence for assigning regional Red List categories to non-evaluated amphibian species, we will also list species with their distribution area entirely situated within the newly proposed European region as well as potential taxonomic changes. Additionally, we will point to the studies published after completion of the last amphibian assessment that could change Red List status of some species in European region. The aim of this presentation is to facilitate discussion among amphibian experts, which will result in the most adequate species listings following IUCN criteria.

**Key words:** European amphibians, IUCN Red List status, regional assessment, updates
Colonization by *Salamandra infraimmaculata* of new breeding sites in xeric habitats

**GAD DEGANI**

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*S. infraimmaculata* is distributed as isolated populations in various habitats under extreme conditions of this genus at the southern border of its distribution in northern Israel. This species is found in danger of annihilation in this area. Its adaption to various types of predictable and unpredictable breeding sites has been studied extensively, since this is one of the most important limiting factors for the conservation of *S. infraimmaculata* on the southern border of its distribution. Colonization of new breeding sites in xeric habitats is an important adaption that is not well described in this species. Many types of human-made breeding sites are used by *S. infraimmaculata*, including sites in which the salamanders colonized every year, were unable to move back and subsequently died. This situation gives opportunity to study the ecological conditions of the colonization of new breeding sites by *S. infraimmaculata*. The salamanders' colonization of human-made breeding sites involve ecological conditions feasible for larvae growth and complete metamorphosis.

**Key words:** colonization, human-made breeding sites, *Salamandra infraimmaculata*
Environmental assessment for the proper management of specimens of *Trachemys scripta* ssp. in relation of blood chemistry and serum protein electrophoresis parameters

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The aim of the study is to compare the data collected from 101 specimens of *Trachemys* ssp.; they were kept in semi-natural condition in an artificial pond at the Ronchi educational center of the WWF (Ms), Italy. The study took place over two years through the execution of serial blood samples, in particular in pre- and post- hibernation periods; all the animals were monitored in the days after the samples. All the samples were performed by the same operator and hemolized or clotted samples have been discarded from final evaluation. All the data collected and the subsequent statistical analysis, associated to a clinical evaluation of the subjects examined, to evaluate the physical and sanitary conditions, allowed to highlight how the management in a controlled environment, with respect the species-specific needs, such as hibernation, affects the elements blood levels. How collected provides important diagnostic and prognostic informations. Further studies are necessary to better evaluate and then compare the possible differences between different types of management, to highlight how good management, both nutritionally and enviromentally, with the respect of annual cycles, can influence the health of these animals.

**Key words:** natural environment, management, hibernation
Consequences of fish introduction and extirpation on populations of metamorphic and paedomorphic newts

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Facultative paedomorphosis in newts and salamanders is a polyphenism that results in the coexistence of two morphs: the paedomorphs that retain gills at the adult stage and the metamorphs that undergo metamorphosis. Paedomorphs are more endangered than metamorphs because they are present in much less populations. As fish introduction is one of the main causes of amphibian decline worldwide, we aimed at determining its consequences on both phenotypes. To this end, we determined occurrence and abundance patterns across aquatic habitats that differ by their current or past presence of introduced fish. The study took place between 2002 and 2015 in Larzac, in southern France, a hotspot for these developmental processes in the palmate newt Lissotriton helveticus. Long-term comparisons were done with historical data collected in the seventies. The results show a low resistance of both phenotypes to fish introductions. Although newts can persist in the presence of fish, they do not reach high population sizes such as in fishless ponds. Moreover, the occurrence of paedomorphs in fish ponds was also much rare. Because of conservation management or natural factors, fish disappeared in many ponds and allowed a high resilience of newts. Their abundance was high and close to control ponds in which fish were never introduced. Paedomorphosis was highly expressed as paedomorphs were found in most of the ponds in which fish were extirpated. From one hand, these results show the detrimental effect of fish introductions on both the common metamorphic phenotype and the endangered paedomorphic phenotype. On another hand, they show that there is hope for their conservation as alternative developmental pathways can reappear even when only the common phenotype persisted in the wild.

Key words: amphibian decline, conservation, fish introduction, invasive species, paedomorphosis, resilience
EPMAC: A reference tool for the evaluation of regional research on amphibians

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In 2011 the first Educative and Participative Monitoring for Amphibian Conservation (EPMAC) event was organized in Southwestern Spain (Sierra Norte natural park). From 2011 to 2015 annually 2 sampling rounds were executed to which over 250 persons have participated. During the monitoring professional, semiprofessional herpetologists and naturalists sample standardized over 100 breeding sites of amphibians distributed over a wide region (approximately 1500 km\textsuperscript{2}). The results are directly made available: species distribution, relative occurrence and abundance of larvae. The results of the first five years of monitoring show the importance of such a reference for the interpretation of other amphibian (inventory) surveys in the region. The relative frequency of occurrence of breeding of many species varies strongly from year to year in most, but not all species. Since 2013 a similar EPMAC monitoring runs in Northeastern Poland (Bialowieza and Narew regions). The results of three year monitoring indicate that also in this region the detection of the various species and larval densities strongly vary from year to year. 2013 and 2014 were years with detection in a low proportion of breeding sites. The observation of this other regions (Estonia, Northern Germany and the Netherlands) stresses the importance of such a reference standardized monitoring. A reference can allow a more accurate interpretation of any survey and research on distribution, relative abundance and occurrence, or habitat. We suggested to develop the EPMAC monitoring over more regions in Europe as a combined tool for education and as a reference for the relative occurrence of larvae and state of the breeding season.

Key words: Amphibian conservation, monitoring, volunteers, Europe
Patterns of Amphibian Community Organisation in Central European Floodplains

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River floodplains are important biodiversity hotspots. They function as primary habitats and dispersal corridors for many amphibian species. Nonetheless, especially in densely populated regions such as North America or Central Europe, most floodplains are heavily degenerated due to river regulations despite their importance for the maintenance of a rich biodiversity. Thus, the conservation need of river floodplains is high, but especially the successful implementation of amphibian conservation actions in floodplains is difficult due to our limited understanding of amphibian habitat requirements. In my study, I identified general patterns in amphibian community organisation in floodplain habitats. I used literature data of 19 Central European amphibian species for 17 life history traits to investigate species ability to cope with floodplain hydrological dynamic and functional groups within communities to better understand habitat requirements. I found four functional groups of species representing floodplain habitats of different hydrological variability, ranging from species specialized to dynamic environments to species generally absent from floodplains. Overall, I found most Central European amphibian species being better adapted to hydrologically highly dynamic environments than to more permanent habitats. My results underline the importance of natural dynamics in floodplains as a major determinant of habitat preferences of amphibian species and therewith for the amphibian community at a given site.

Key words: amphibians, dynamic floodplains, community organisation, functional groups, Central Europe, conservation
Computer vision-based method for recognition of individual patterns in amphibians

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Effective monitoring of demographic parameters and life history data in amphibian populations usually requires marking techniques adjusted to the particular target species. Methods used to identify individuals can be permanent, temporary, and/or individual-specific, and their use depends on the aim of the study. Capture-recapture methods present the most reliable source to provide precise and relatively long-term population estimates. Individual recognition may be obtained either by artificial marking or in case of availability by using an animal's natural markings. Artificially marking of wildlife can stress individuals, cause injuries, and affect individual performance. Moreover, some marks or tags may not be enduring enough to be of use leading to potentially impaired demographic patterns. Unlike invasive marking methods, photographs do not mutilate the animal and therefore offer preferable alternative for very sensitive or endangered species. The application of photography for animal identification concerning stable and individual, natural marks has been successfully used in studies on several vertebrates. Regarding amphibians, numerous studies reveal that color patterns serve as a highly suitable feature for individual identification in a wide range of species. Photo libraries of relatively small amphibian populations can be examined manually, whereas the expenditure of time increases extensively when surpassing 100 registered individuals. To circumvent this problem we present a composition of a handy sized hardware and software which facilitates individual amphibian identification in the field. Here, a modern approach from the field of computer vision and machine learning allows fast and reliable recognition of individuals within existing population databases. Results from comprehensive tests deliver outstanding classification accuracies, suggesting further application of non-invasive, vision-based marking techniques.

Key words: individual identification, capture-recapture methods, amphibians, machine learning, computer vision
The evolutionary relationships of Malagasy scincines – a potential future model clade to study limb evolution in squamate reptiles

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The evolution towards a limb-reduced morphology has arisen independently in various clades of squamate reptiles. The Malagasy scincines comprise a clade of approximately 60 species, which show a striking variation in the way and degree in which limbs have convergently regressed within several fossorial lineages. These include evolutionary trajectories towards fully limbless morphologies, but also involve intermediately regressed morphologies with extant species showing only rudimentary hindlimbs, or on the contrary, showing only forelimbs. These patterns of limb reduction are most apparent in the miniaturized species of the genera Paracontias, Voeltzkowia and Sirenoscincus, but may also concern other species currently recognized in the genera Amphiglossus, Pygomeles and Pseudoacontias. The relationships among most of these fossorial lineages remain unresolved, and patterns of limb evolution in these enigmatic burrowing species have not yet been examined in detail. Here we summarize our recent attempts to elucidate the phylogenetic relationships of Malagasy scincines through an improved taxon sampling and a more extensive molecular dataset consisting of nine nuclear and three mitochondrial markers. In addition, we performed CT-scans that highlight the appendicular osteology of several key taxa of limb-reduced skinks. The integrated molecular and morphological data support a new phylogenetic hypothesis for Malagasy scincines suggesting various taxonomic changes, and reveal a potential rapid process of limb reduction that occurred at a notably higher rate than usually observed in other lizard radiations. Despite remaining phylogenetic uncertainty and unavailable molecular samples for several species, we anticipate that Malagasy skinks could serve as a highly interesting model clade for future studies to body-form evolution in squamate reptiles.

Key words: Madagascar, Scincidae, Convergent evolution, Limb reduction, Morphology, Molecular phylogenetics
Niche Partitioning at Local and Regional Scale in the North African Salamandridae

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The composition of Urodela assemblages is regulated by macro- and microclimatic conditions and by the interactions established between ecologically analogous species. Species of the North African Urodela are distributed unevenly; some species have large ranges whereas others occur in fragmented populations or in restricted ranges. We examined the niches occupied by these species to determine the factors that regulate their range patterns. The niches were examined at two spatial levels; regionally, using climatic and vegetation cover data, and locally by studying the selection of aquatic habitats. Our results indicate the Salamandra algira and North African Pleurodeles species are segregated along a thermal and vegetation cover axis, although with considerable overlap. The fragmentation observed in the distribution of the North African Urodela is caused by prevailing arid conditions in the region. The three Pleurodeles species appear under similar climate conditions and mostly use temporary ponds to breed. There is some overlap comparing species from both genera in the selection of breeding habitats, but the North African Pleurodeles species occur in ponds with higher water temperatures than do S. algira. Preserving temporary ponds, streams, and springs is essential for conserving these species, particularly under the semiarid conditions that favor the fragmentation of their populations.

Key words: allopatric species, niche conservatism, Pleurodeles, Salamandra
Niche evolution in European Hydromantes: mismatches between macroecological and fine-scale analyses

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Ecological niches are increasingly analyzed by combining distribution records with broad-scale climatic variables (macroecological approach), however interactions between species and their environment often occur at fine scales. The idea that macroscale analyses correctly represent fine-scale processes relies on the assumption of scale invariance, but tests of this hypothesis are scarce. We compared the macroecological and the fine-scale (microhabitat) approaches, by analyzing the niches of all species of European plethodontid salamanders (Hydromantes ambrosii, H. flavus, H. genei, H. imperialis, H. italicus, H. sarrabusensis, H. strinatii, H. supramontis). Macroecological niches were characterized on the basis of species presences and bioclimatic variables gathered over broad scales, while fine-scale analyses relied on presence records obtained in the field, and on the microhabitat selected during the stressful summer conditions. First we showed that microhabitat parameters represent well the operational conditions of individuals. Both the microhabitat and the macroecological approaches identified niche differences among species, but the correspondence between micro- and macroecological niches was weak. Strikingly, species identified as dry-tolerant by microhabitat analyses (e.g. H. strinatii, H. ambrosii) were associated with the wettest climates in macroecological analyses. When exploring niche evolution, the macroecological approach suggested a close relationship between niche and phylogenetic history, but this relationship did not emerge in fine-scale analyses. The apparent pattern of niche evolution was likely the byproduct of related species having nearby ranges. The environment actually experienced by terrestrial salamanders is actually more heterogeneous than what is apparent from macro-scale predictors, and a better combination between macroecological and fine-grained data may be a key to obtain robust ecological generalizations that can really help us to address the consequences of global changes.

Key words: niche evolution, Plethodontidae, niche similarity, caves, microhabitat, climate
How to identify species? An integrative approach to delimiting species in the threatened Middle Eastern mountain newts

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The species plays a fundamental role in zoological fields such as ecology, evolution, biogeography, and conservation. Therefore, objective and clear-cut recognition of species is paramount. Molecular methods, especially barcoding, have become a popular tool to identify and delimit species and facilitated traditional morphology-based species recognition. However, following the plea for an integrative taxonomy, more lines of evidence should be incorporated when identifying species. While an increasing number of studies use ecological niche modelling (ENM) within an ecological and evolutionary framework, there are only few examples of ENM used to support taxonomy. The Middle Eastern mountain newts (genus Neurergus) currently comprise six taxa whose taxonomical status has been under debate. We aim to delimit species within this genus by combining different, independent lines of evidence. These include DNA barcoding (using the two mitochondrial genes 16S rRNA and COI), multi-locus species trees evaluated using Bayesian species delimitation under different priors, morphological overlap analyses through n-dimensional hypervolumes of morphometric and colouration characters, and comparison of n-dimensional hypervolumes of the ecological niches using PCA-derived variables of environmental data obtained from occurrence records. The consensus of all methods suggests four species within Neurergus. However, the conflicting results regarding species delimitation between the used methods underline the necessity of combining multiple approaches within an integrative framework. Especially in threatened taxa like Neurergus, focussing on one method can mislead taxonomy and consequently hinder species-based conservation assessments. Different realised niches indicate specific divergence, as physiological tolerances that define the niche evolve during or trigger speciation. Accordingly, ENM provides a further, well applicable tool to test species hypotheses, but needs to be interpreted with caution.

Key words: Ecological niche modelling, integrative taxonomy, n-dimensional hypervolume, Neurergus, species delimitation
The big picture: integrating novel and traditional approaches to herpetofauna surveillance

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A wide range of different survey approaches are used to study amphibian and reptile populations; these reflect the differing motives behind the survey, the specific interests of the surveyors themselves and the resources and analytical capabilities of the support network. ARC has developed a ‘framework’ document to help better understand the data needs for a range of conservation assessments and to aid in targeting conservation actions. This was loosely based around the four pillars used in the European Union’s Habitats Directive for assessing conservation status, though deliberately using a loose interpretation such that key conservation evidence needs could be met. The framework considers four key population parameters which are:

1. Range (and distribution)
2. Population status (including abundance and viability)
3. Habitat (extent and quality)
4. Long term prospects – including threats and positive conservation actions.

We will look at examples of different approaches and techniques and how these may be developed to assist surveillance work and to help determine of conservation goals and targets, considering how this links to the concept of a Favourable Conservation Status of species. While differing methods may be favoured for specific applications, e.g. for robust trend analysis versus understanding local distribution, we advocate that a combination of approaches will provide a more cost effective and sustainable solution to meeting conservation needs. These will involve developing volunteer and amateur programmes alongside professional surveys, ensuring effective data management and exploiting new techniques such as environmental DNA sampling and GIS analysis alongside traditional field survey methods. While we strongly advocate the use and development of species distribution modelling, particularly alongside improving systems for obtaining environmental data (notably through remote sensing) we caution that this should be used to support, and to be supported by, an effective programme of field survey.

Key words: survey, monitoring, predictive mapping, volunteer engagement, Favourable Conservation Status
Genetic diversity within the adder (Vipera berus) in Italy and surrounding regions

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The use of genetic markers has led to a rapid increase of the number of species during the last three decades, by unveiling the presence of cryptic species and complex genetic structure within numerous species. In the adder (Vipera berus), the mtDNA analyses confirmed the validity of V. b. bosniensis, but highlighted the presence of a genetic clade distributed in the southern part of the Alps (Italy, extreme southeast Switzerland, a large part of Austria and Northern Slovenia). However, no investigation has been conducted on the morphology or the distribution of this genetic clade. Moreover, it is not clear if the genetic clades are genetically isolated – and should consequently be considered as different species – or there is gene flow between them – and they should be considered just as local historical genetic variations. This talk will present the results of some morphological and genetic works on the Italian (or Alpine) genetic clade, with some astonishing results regarding the number of species inhabiting the Alps and related biogeographical implications.

Key words: Vipera berus, Alps, cryptic species, speciation
Biogeography of *Agama boulengeri*: using spatial and genetic tools in a multi-scale assessment of a Sahara-Sahel lizard

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Desert biomes are one of the least studied on the planet, and patterns of biodiversity distribution and processes controlling evolution are still mostly known only in a very broad way, particularly in regards to herpetofauna. Some biogeography studies are available, but are mostly focused on general phylogeographic inferences. Many subjects requiring fine scale analysis have been only lightly assessed until now, for instance gene flow, contact-zone and corridor dynamics, landscape connectivity, or the influence of climate. Here we present a biogeographic analysis of *Agama boulengeri*, one of the promising model taxa for studying evolutionary processes shaping diversity in Sahara/Sahel. From near 400 georeferenced samples, 261 covering the species’ distribution were used for phylogenetic reconstruction and population analyses. 2+2 mitochondrial and nuclear gene fragments and 23 microsatellite loci were analysed. Geostatistics tools were then used to infer the species’ extent of occurrence, past potential distribution and dispersion routes, and to analyse landscape features related to population connectivity. Results indicate humid-arid cycles and the changing landscape played a major role in shaping biodiversity patterns in the region. Highly divergent mitochondrial lineages occur parapatrically in different mountain systems, but microsatellite data indicate present geneflow occurring among them. Past distribution models show the lizards probably persist in the mountains during both arid and humid phases. Landscape connectivity analyses indicate rocky areas work as corridors for dispersion while sandy or grassy areas like temporary river beds have a barrier effect. In conclusion, dispersal behaviour and habitat availability might be playing an as important a role as climate in the distribution of genetic variability and the maintenance of millions of years old genetic structure.

**Key words:** Desert, sky-islands, diversification, phylogeography, ENMs, landscape connectivity
The *Boaedon fuliginosus* complex – phylogenetic analyses

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Different morphometric parameters of at least 400 specimens of the *B. fuliginosus* complex from all over the African continent were recorded and compared. DNA data of 50 specimens were analyzed from fresh and preserved material. Cladistic analyses using 16S and 12S RNA were made. First results indicate from both morphology and molecular data at least 8 clades with two new species. We propose to revalidate *Boaedon mentalis* and *capensis* from synonymy and consider them as full species. Distribution data of the clades will be presented in a map. Their biogeography will be discussed.

**Key words:** Serpentes, Lamprophiidae, systematics, morphology, molecular biology, distribution
Post-release survival of Hungarian meadow vipers (*Vipera ursinii rakosiensis*)

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Hungarian Meadow Viper Conservation and Breeding Centre was started in 2004 with 16 adult individuals, collected from 6 different populations. By the end of 2014 number of vipers bred reached 1900 individuals. Reintroduction of vipers was started in 2010, releasing 400 snakes into reconstructed habitats of Kiskunság and Fertő-Hanság National Parks. Snakes were released by removing them together with artificial burrows, used in outdoor terraria of the Breeding Centre. During regular monitoring, besides surveying the release sites we checked these burrows by using pipe-camera. In the release sites vipers were spotted 255 times and in 147 occasions photos taken were accurate enough for individual identification, belonging to 69 individuals of which 43 were recaptures, concluding about a minimum 25% survival rate. In order to develop a remote tracking method, pre-programmed radio-tags with a detection range of 100-150m were implanted surgically into the abdomen of vipers. These tags also operate as temperature loggers, recording data every five minutes for a year-long operation period. After successful testing during 2010 and 2011, we started releasing tagged individuals in 2012. Over the past 3 years we were able to track 20 vipers and recover 14 tags, gaining recorded temperature data as well. Generally vipers tend to move short distances and they preferred to use natural burrows. Large proportion of known predation is blamed on aerial predators, with November and March being the most dangerous months for vipers, as they tend to spend more time on the surface, while basking. We also installed camera traps since 2012 and recorded frequent presence of such possible predators like fox, badger, common buzzard and wild boar.

**Key words:** Hungarian meadow viper, captive breeding, reintroduction, radio-telemetry
Road density and wetland context alter population structure of a freshwater turtle

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Many species of freshwater turtles are vulnerable to habitat fragmentation and traffic mortality caused by roads and vehicles. The common long-necked turtle (\textit{Chelodina longicollis}) is an Australian freshwater turtle that frequently moves between wetlands and so populations may be negatively impacted by road effects. We assessed the relationship between \textit{C. longicollis} and road density and landscape variables within populations inhabiting 20 wetlands distributed throughout the Melbourne region in southern Australia. The size frequency distribution of \textit{C. longicollis} at sites surrounded by high road densities was dominated by larger individuals, but there was no difference in frequency of juveniles between high and low road density sites. Regression models revealed a clear positive relationship between road density and carapace length (CL) of \textit{C. longicollis}; the mean CL at a site with the highest road density was predicted to be 23\% greater than mean CL at a site surrounded by no roads. Female CL was also positively related to road density. There was a clear positive relationship between wetland age and CL, although this relationship was not as strong. While there was no relationship evident between road density and the proportion of female \textit{C. longicollis} at a site, more females were captured at smaller ephemeral waterbodies surrounded by a high proportion of green open space and located near drainage lines. We did not find evidence of sex-related differences in road effects. Our results suggest that roads may be affecting \textit{C. longicollis} in the study area, but the direct cause of any effects is difficult to identify. We recommend that management strategies for \textit{C. longicollis} and other freshwater turtles protect both wetland and terrestrial habitats, and consider use of under-road tunnels to assist turtle movement.

\textbf{Key words:} \textit{Chelodina longicollis}, demography, reptile, road effects, urbanisation, urban wetlands
Disentangling the *Calotes mystaceus* complex

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The widespread agamid lizard *Calotes mystaceus* Duméril & Bibron, 1837 was commonly regarded as a single species occurring throughout Southeast Asia. The recent discovery of a superficially similar but distinct species (*Calotes bachae*) from southern Vietnam initiated a comprehensive investigation of the formerly recognized species *C. mystaceus* sensu lato. A combination of morphometric, genetic and multivariate spatial methods was used to dismantle a total of five charismatic species. To assess variation within the *Calotes mystaceus* complex, an extensive morphometric dataset (23 mensural and 11 metric characters) of more than 100 specimens from all across its distributional range was compiled. The dataset was analyzed using classical multivariate techniques. In addition, samples for molecular genetic analyses were collected from a representative subset of specimens. Samples were analyzed using the fast evolving cytochrome oxidase I (COI) and mitochondrial 12S rRNA (12s) genes to identify even minor genetic differences between populations. Including our discovery of *Calotes bachae* in 2013, we were able to dismantle four new species formerly unrecognized and included in the widespread taxon *Calotes mystaceus*. Finally, we used multivariate spatial approaches to compare the environmental niches of the newly discovered taxa. In conclusion, our integrative use of diverse methods enabled us to formally describe and hence, unravel the highly underestimated diversity in this charismatic group of agamid lizards.

**Key words:** Agamidae, cryptic species, molecular genetics, morphometrics, species distribution modelling, systematics
Molecular taxonomic identification and determination of hybrid ploidy levels among European water frogs (*Pelophylax*) in Hungary

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The western palearctic water frogs in the genus *Pelophylax* include twelve species with partially overlapping morphology. Some of these species can form hybridogenetic complexes. Such type is the *Pelophylax esculentus* complex formed by two sexual species: *Pelophylax ridibundus* (genotype RR), *Pelophylax lessonae* (genotype LL), and their hybridogenetic hybrid *Pelophylax esculentus* (genotype LR, LRR or LLR). This study was conducted in the Hortobágy National Park which is the largest contiguous alkaline steppe in Europe covering 80,000 hectares. Altogether 164 water frogs were captured during the years 2012-2014 from three different types of wetland habitats. Due to the high rate of hybridization and partially overlapping morphological features among water frogs we used molecular taxonomic techniques to distinguish between *P. ridibundus*, *P. lessonae* and the hybrid. This method is based on the PCR products size differences from the amplification of the serum albumin intron-1 (SAI-1) gene fragment. We also used 15 microsatellite loci to distinguish between the three different genotypes in hybrid individuals. Based on fragmentary analysis we detected the presence of all the members of *P. esculentus* complex. From 164 samples we determined the taxonomic status of 101 *P. ridibundus*, 1 *P. lessonae* and 62 *P. esculentus* individuals. Based on microsatellite analysis we found that all the possible genotypes were presented in hybrid individuals.

Key words: *Pelophylax esculentus* complex, genotyping, microsatellites, hybridogenesis, population systems
Using environmental DNA to find the bastards; a case study on Northern crested newt and the invasive Italian crested newt in the Netherlands

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Alien invasive species (AIS) are one of the major causes of biodiversity loss. Once an AIS becomes established, costs of control can be extremely high and complete eradication is not always achieved. Early detection plays a crucial role. However, detection of AIS at low densities is often limited with traditional monitoring methods. Moreover, in cases where AIS hybridize with related native species, morphological identification is often unreliable. Here we present the results of a case study on crested newts from the Netherlands. The Northern crested newt (Triturus cristatus) is the only native crested newt species in the Netherlands. The Italian crested newt (Triturus carnifex) has been introduced in the Veluwe nature reserve on a location where T. cristatus was absent. Recent studies, based on traditional methods (dip nets and fykes), showed a range expansion of T. carnifex into areas where T. cristatus was present. Genetic studies based on tissue samples and nuclear DNA showed an even further expansion of T. carnifex and genetic admixture between the two species. After colonization of new areas, initially T. carnifex occurs at very low densities which make them hard to detect. We therefore conducted a successful pilot study to investigate the potential use of environmental DNA (eDNA) to monitor the distribution of both T. carnifex and T. cristatus. Next, a large area was screened to determine the actual range of both species. We will discuss the results of this study and the advantages and limitations of the eDNA method compared to traditional methods and other genetic methods.

**Key words:** environmental DNA, Triturus carnifex, Triturus cristatus, hybridization, invasive species; monitoring
The effect of food and vitamin D availability on behavioural consistency of male Carpetan rock-lizards (*Iberolacerta cyreni*)

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Behavioural consistency is expected to affect fitness in a negative way intuitively by constraining the individual behavioural repertoire. In contrast to this, consistency both within (animal personality) and across behaviours (behavioural syndrome) is observed in a wide variety of taxa. Hence, one of the main goals in behavioural ecology is to understand the evolutionary and developmental factors underlying consistent between-individual differences in behaviour. One possibility is that individual state and behaviour are linked, and thus state-behaviour feedback loops can explain the emergence of behavioural consistency. For instance, body condition is expected to have a strong effect on individual behavioural strategies. It is known that the vitamin D component in the femoral secretion of male Carpetan rock-lizards (*Iberolacerta cyreni*) act as an honest sexual signal, since only males with better body condition can afford secreting vitamin D at a high rate. Hence, vitamin D and available energy are both expected to affect their behavioural consistency. In our present work, we studied the effects of food and vitamin D manipulation on the activity and risk-taking of 60 male *I. cyrenis* during the mating season of 2014. We applied a full factorial experimental design with high vs. low food treatments and vitamin D supplementation vs. placebo treatments. We discuss the treatment effects on lizard behaviour by focussing on two components of individual behavioural variation: individual mean behaviour and within-individual behavioural variation based on ten (activity) and seven (risk-taking) repeated assays.

**Key words:** *Iberolacerta cyreni*, behavioural consistency, vitamin D, food availability
Severe over-collection, habitat loss, and other anthropogenic hazards contribute to tremendous population declines in Asian turtles. As a result, most Southeast Asian chelonian species are threatened by extinction. Taxonomic research on widely distributed taxa is considered a priority for future research to identify hitherto unrecognized cryptic species. Southeast Asian Snail-eating Turtles of the genus *Malayemys* are heavily exploited geoemydid turtles inhabiting a variety of natural and artificial freshwater habitats across Southeast Asia.

While individuals from the western portion of the distributional range (Chao Phraya and Mae Klong River Basins in central Thailand) were recently recognized as a separate species (*Malayemys macrocephala*), the eastern populations inhabiting eastern Thailand, southern Laos, Cambodia, and southern Vietnam retained the name *M. subtrijuga*. However, distinction of the two species is solely based on few morphological differences and is still controversial.

We combined molecular and morphometric data to assess the phylogenetic diversity and re-assess the taxonomy within this widely distributed genus. Comprehensive analyses of the mitochondrial ND4 and cytochrome *b* genes revealed three distinct lineages, which do not match any geographic pattern. However, these lineages are concordant with results of morphological analyses using 30 morphometric characters. Our preliminary findings suggest the existence of three distinct taxa corresponding to the two previously recognized species *M. macrocephala* and *M. subtrijuga*, and a previously unrecognized third taxon.

**Key words:** Field study, genetics, phylogeography
Identification of syntopic anuran species at early tadpole stages: could morphometric analysis resemble molecular genetics?

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Many European frogs and toads are relatively secretive species and, except breeding season, adults rarely can be seen during time-restricted fieldwork. On the contrary, their tadpoles are easy to record. For the brief biodiversity assessment, it is important to perform quick and accurate taxonomic identification of tadpoles. Genetic methods are still costly and thus cannot be routinely applied. Therefore, we tested suitability of body shape analysis for taxonomical distinction among tadpoles of brown frogs and the common toad. Morphometric characters were chosen based on relevant literature and included eye distance, head height, eye diameter, tail length, tail muscle height, tail height, mouth length, head width, head length and body length. Measurements were standardized by body length. We compared tadpole samples classified as hatchlings collected simultaneously at three different localities in Serbia, in habitats known as breeding sites shared by brown frogs and the common toad. Independent taxonomic identification using DNA barcoding verified that samples from localities 1, 2 and 3 belong to Rana dalmatina, R. temporaria and Bufo bufo, respectively. The results of linear (traditional) morphometric analyses suggested that relative head length and relative head width could be good discriminative characters between tadpoles of these two Rana species and B. bufo. The relative tail length could be used for distinguishing tadpoles of two analyzed brown frog species. However, for the further development of the identification procedures for tadpoles of species of concern, it is essential to involve geometric morphometry and to analyze different larval developmental stages.

Key words: tadpoles, brown frogs, common toad, taxonomic identification, morphometry
A report on variation of breeding phenology in common toad

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Long–term studies on breeding phenology of the Common toad (Bufo bufo) in the western Europe demonstrated that ambient temperatures have an effect on the arrival time of adult toads to the breeding place. We implemented the same study design on the records on Common toad breeding phenology collected from 2001-2003 and 2011-2015 in Southern Europe (Belgrade, Serbia). Variables analysed were: 1. first day when male(s) were detected in the pond, 2. main toad arrival to the pond, 3. peak of breeding activity, 4. last day when the adult(s) were recorded in the pond, and 5. duration of the breeding season (number of days counted from the first to the last day when the adult toads were seen in the pond). We tested relation between those variables and two ambient temperatures: a) mean daily temperature over 40 days preceding the main toad arrival to the pond, and b) mean daily temperature over period of 5 to 60 days preceding the main toad arrival.

Strong positive correlation was found between variables 1-3 (r varied from 0.888 to 0.999; p varied from 0.003 to 0.000). Variable 4 was positively correlated to variable 5 (r=0.74, p=0.04) and negatively to variable b (r=-0.75, p=0.03). Correlations between variables 1-5 and two ambient temperatures were not detected.

The highest mean temperature (9.5°C) for the 40 days preceding breeding activity was recorded in 2002 (an 'average' breeding year for toads). The lowest mean temperature (0.8°C) was recorded in 2003 (a 'late' breeding year).

Though we did not detect an influence of two ambient temperatures on the dynamic of toad breeding activity on analysed locality, the results indicated a slight shift toward earlier start of the breeding season and faster reaching of the peak of breeding activity.

Key words: Breeding, Bufo bufo, temperature
Where does variation come from? Linking patterns of morphological, genetic, and environmental differentiation and diversity across space in wall lizards

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Variation is a central concept in evolutionary biology, as it represents the raw material for the action of selection. Phenotypic variation among populations of a species reflects the combined result of random drift and selection, and it influences the capacity for adaptation to changing environmental conditions. Due to their high phenotypic and genetic variability, and their ecological flexibility, \textit{Podarcis} wall lizards are an intriguing model for exploring patterns of variation. For this purpose, we collected data from nine populations of \textit{P. bocagei} in Portugal (N=305) and nine populations of \textit{P. vaucheri} in Morocco (N=285). For each individual we recorded biometric and pholidotic traits, head shape using geometric morphometrics, and we genotyped a total of nine microsatellite loci. We then used multivariate approaches in a geographically explicit framework to address the following questions: 1) Is there an association between morphological and genetic variation across populations? 2) Are morphological and genetic traits spatially structured? 3) What is the relevance of environment for determining these spatial patterns? Mantel tests did not reveal a significant association between morphological and genetic differentiation across populations of neither species. Indeed, Inverse Distance Weighted interpolation of morphological and genetic distances revealed that both traits exhibit spatially structured variation, which is however largely trait-specific. Generalized Dissimilarity Modeling indicated that geographic distance and environmental variation are involved in shaping morphological and genetic variation in \textit{P. bocagei} and \textit{P. vaucheri}, but also revealed that different ecological factors are relevant in each species. These results support the idea that morphological and genetic variation within \textit{Podarcis} species respond to different evolutionary processes, which may also vary across species. These microevolutionary patterns are in accordance with the frequent dissociation between morphological and genetic differentiation observed across \textit{Podarcis} species, and they provide hints into how within-species variation may translate into macroevolutionary diversification.

**Key words:** diversity, biometry, microsatellites, Mantel test, interpolation

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Time data and the morphologic characteristics of embryonic development of the *Hyla savignyi* from fertilization to 25th stage are presented, following Gosner’s generalized table. Fertilized egg obtained from two amplexus specimens that were gathered in breeding season from Northern Cyprus Kalkanlı region. Samples were set in 3 groups and were examined and photographed every 10 minutes. Embryos hatched at stage 20 or 21 that come up to 3-4th day after fertilization. Embryonic development of *H. savignyi* is about 157 hours (7days) at 21±1 °C. Cleavage is unequal holoblastic. This study provides a comparison of embryonic development time data with *H. savignyi* and *H. arborea* which shows a longer embryonic development period in *H. arborea* (211.30 hours=9 days) than *H. savignyi*. Development completed in a shorter period in *H. savignyi* and this is resulting an advantage to adapting in hot zone.

**Key words:** Hylidae, *Hyla savignyi*, embryonic development
Evaluation of Habitat Suitability Index for Great Crested Newt (*Triturus cristatus*) in central Poland

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Habitat Suitability Index (HSI) for Great Crested Newt (*Triturus cristatus*) is commonly used across Europe as a tool in species field inventory. The HSI is a geometric mean of ten suitability indices describing different habitat conditions of pond and its surroundings. The aim of this study was an evaluation of this method for Central Poland’s environmental conditions (lowlands). The HSI was determined for 168 ponds in the vicinity of Warsaw – Central Poland (in the territory of Mazowiecki and Chojnowski Lanscape Parks and Kampinoski National Park). There was no correlation between HSI and occurrence of great crested newt, although mean HSI for ponds with news presence was relatively high (0.72 – that corresponds to good habitat quality). All indices were analysed to verify their contribution to entire index. Most crucial ones were respectively: terrestrial habitat quality, nearest pond presence, pond surface area and shade of surrounding trees. Less important for overall value of index were permanence of the pond (number of years pond dries up totally in five years), fish and fowl presence, macrophytes cover and water quality. Further statistical analysis will also be discussed. The results show moderate usefulness of HSI for great crested newt inventory. Further studies should be conducted to evaluate this method in Poland’s environmental conditions.

**Key words:** Great crested newt, HSI, Kampinoski National Park, Mazowiecki Landscape Park, Chojnowski Landscape Park
Amphibian road mortality mitigation in the Czech Republic

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Amphibian mortality on roads is a big problem of nature conservation in the Czech Republic. Many individuals are killed every year when migrating to their breeding places. The most affected species is Common Toad (*Bufo bufo*) but Common Frog (*Rana temporaria*) or Newts also face serious difficulties in some areas. Thus specific activities are needed.

Nature Conservation Agency of the Czech Republic is a governmental body responsible for nature conservation all around the country. Mitigation of road related mortality is one of the tasks we are dealing with. Our main role is to coordinate and prioritize needed activities and support NGOs that are performing transfers on the most important road sections.

We prepared an on-line map showing all listed dangerous road sections. This map is based on a database which is updated every year after spring migration season. There are currently 550 sections, divided into 6 groups based on the severity of the problems. Two categories include sections with high mortality. There are higher tens of amphibians killed in Risky sections (78) and hundreds in Critical ones (9) every year. On the other hand there are three types of sections where mortality is minimal. It is due to human made transfers in Secured sections (103) or building of tunnels with barriers in Solved sections (13). Small number of amphibians is crossing roads in Minor sections. Last category is called Data deficient.

This map and database are the basis for next road mortality mitigation steps. Building of tunnels is a good solution where higher hundreds or thousands of amphibians are migrating. However, yearly human made transfers are better when construction is not possible due to different obstacles. Our goal is to lower the number of Critical sections to zero in upcoming years and to solve the problem wherever it is possible.

**Key words:** road, mortality, mitigation, database
Evolutionary relationships and phylogeography in desert lizards from North Africa: the Acanthodactylus scutellatus species complex

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Geological and paleoclimatic events are two of the most important drivers of evolutionary processes in nature. These are poorly studied phenomena in North Africa, despite its great diversity of habitats, heterogeneous landscapes, and complex climatic and geological histories. Modern biodiversity patterns of the Sahara-Sahel likely resulted from strong fluctuations in climate and land-cover. Acanthodactylus scutellatus species complex comprises important elements of the herpetofauna of arid ecosystems in North Africa. Despite their remarkable diversity, the taxonomy within the complex is controversial, and with the exception of morphological data, little is known about these organisms. Observations of individuals with intermediate morphotypes suggest hybridization between taxa. The present study aims to: 1) infer phylogenetic relationships within this group and identify major lineages; 2) understand the phylogeographic patterns of north-western African lineages; 3) infer contemporary gene flow in a contact zone in Mauritania. Phylogenetic and phylogeographic analyses were based on about 450 specimens sequenced for both 12S and Cyt-b mitochondrial genes (756 total bp), and for the C-mos nuclear gene (513 total bp). About 210 individuals of the contact zone were genotyped for 15 microsatellites. Our results show a mismatch between recovered major lineages and current systematic, highlighting the necessity of a taxonomy and systematics revision. The genetic structure found is geographically coherent and can be explained by past oscillations in climate and desert extent. Genotyping analyses confirmed the historical lineages found in the studied contact zone and show absence of gene flow between them, with no hybrids detected despite the large sampling and broad geographic coverage. Lack of observed gene flow raises questions about the putative role of habitat selection and reproductive barriers in preventing hybridization. Finally, these results provide insights on the species boundaries, relationships, history and diversity of this group, and contribute to the knowledge about gene flow in the Sahara-Sahel.

Key words: Acanthodactylus scutellatus, North Africa, phylogeny, phylogeography, gene flow
Trapped within the city: the effects of recent isolation on population structure and demography of *Salamandra salamandra* in Oviedo

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Habitat loss and fragmentation produced by the growing urbanization have a profound impact on biodiversity, although some species managed to survive within cities. Fire salamanders (*Salamandra salamandra*) of the city of Oviedo (Spain) constitute a remarkable example of adaptation in urban areas. These populations evolved to viviparity during Pliocene-Pleistocene times, which allow them to inhabit harsher environments, such as cities. In Oviedo, small groups of individuals persist within parks or small gardens, being hypothetically isolated. The city has been growing since the construction of ecclesiastic buildings surrounded by an historical wall (VIII century). Thus, it is expected that genetic differentiation is higher in urban populations located inside walls due to a longer period of isolation. The reduced habitat and small population sizes, allied with available information regarding historical urban development, makes Oviedo a natural laboratory to test the above hypotheses.

We genotyped (15 microsatellites) 285 individuals from locations throughout the city. We aim to: (1) analyse the levels of population structure pattern; (2) assess genetic diversity and inbreeding values across sampled sites; (3) estimate demographic parameters; and (4) evaluate the effects of recent isolation, genetic drift and demography on current genetic patterns in Oviedo. Preliminary results indicate a strong genetic structuring across most sampled populations, although there is not a clear structuring and genetic diversity patterns regarding populations inside and outside walls. Nevertheless, one population within city walls exhibited the highest genetic differentiation (mean pairwise $F_{ST} > 0.15$) and the lowest genetic diversity values. Population effective sizes ($Ne$) were lower within walls (mean $Ne = 14.7$) comparatively to outside walls (mean $Ne = 111.7$), although $Ne$ seemed to be correlated to patch area. The results obtained have the potential to contribute to a better understanding of the effects of recent isolation and other demographic parameters on genetic patterns of natural populations.

**Key words:** *Salamandra salamandra*, habitat isolation, microsatellite, genetic drift, population effective size, demography
Hybridization masks speciation in the evolutionary history of the Galápagos marine iguana

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The effects of the direct interaction between hybridization and speciation—two major contrasting evolutionary processes—are poorly understood. We present here the evolutionary history of the Galápagos marine iguana (Amblyrhynchus cristatus) and reveal a case of incipient within-island speciation, which is paralleled by between-island hybridization. In-depth genome-wide analyses suggest that Amblyrhynchus diverged from its sister group, the Galápagos land iguanas, around 4.5 million years ago (Ma), but divergence among extant populations is exceedingly young (less than 50 000 years). Despite Amblyrhynchus appearing as a single long-branch species phylogenetically, we find strong population structure between islands, and one case of incipient speciation of sister lineages within the same island—ostensibly initiated by volcanic events. Hybridization between both lineages is exceedingly rare, yet frequent hybridization with migrants from nearby islands is evident. The contemporary snapshot provided by highly variable markers indicates that speciation events may have occurred throughout the evolutionary history of marine iguanas, though these events are not visible in the deeper phylogenetic trees. We hypothesize that the observed interplay of speciation and hybridization might be a mechanism by which local adaptations, generated by incipient speciation, can be absorbed into a common gene pool, thereby enhancing the evolutionary potential of the species as a whole.

Key words: Restriction site-associated DNA (RAD) sequencing, single-nucleotide polymorphisms, El Niño, volcanism, introgressive hybridization, morphometrics
Predator cues and risky habitats affect foraging activity in salamanders

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Predator-prey interactions are among the most important interactions occurring in natural habitats and a major driver of a considerable number of phenotypic traits. Predators may affect prey through consumption that kills or injures prey (consumptive effect) or through several other factors (nonconsumptive effects) altering prey morphology, life histories and behaviour. Nonconsumptive effects may be as important as consumptive effects in determining prey populations dynamics. In this study, we aimed to disentangle the role played by predator occurrence like dragonfly larvae on the spatial activity of the fire salamander larvae, considering larvae originating from habitats with very different conditions like epigeous streams and hypogeous caves. We exposed larvae from caves and streams to chemical cues of the dragonfly *Cordulegaster bidentata* and of damaged conspecifics, at the newborn stage and after 40 days of rearing in safe and different risky conditions. We found that foraging activity dynamics like distance moved, average velocity while moving, movement frequency and spatial distribution depended on whether predator cues occurred and on whether larvae were reared in conditions where predation risk was present. In the occurrence of dragonfly risk cues and when reared in risky conditions, salamander larvae moved less and with a lower average velocity. Moreover, larvae reared in risky conditions exploited less central sectors. Cave larvae generally moved less than stream larvae, decreased more their movements with predator cues occurrence and decreased more their velocity while moving when reared in risky conditions. These results provide interesting insights of the pressures acting on the colonization of different environments like caves and help understanding the interplay of important predators of streams and springs, such as dragonflies and salamander larvae.

Key words: behavioural ecology, cave, foraging, predation, salamander
Assessing the risk of pesticide exposure for reptile species occurring within the European Union

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Habitat loss associated with environmental pollution are suggested to be the main thriving factors for reptile declines in industrialized countries. Although reptile diversity is highest in tropical regions, it is necessary to also focus on subtropical and temperate regions. The reason is that industry-induced environmental pollution in general has a high impact on wildlife, such as in the EU. Though many species within the EU benefit from protection thanks to the Habitats Directive, no special consideration is given to possible detrimental effects of pesticides on population declines. This is in particular remarkable as many effects which may lead to a diversity loss have already been identified. We conducted an exposure assessment for all reptile species occurring within the EU for which enough data were available, as a means to conduct pesticide risk assessments. For this purpose, we calculated the proportions of land use with regular pesticide applications within the known geographical distributions of each species. By using three evaluation factors (contact probability, uptake intensity and pathways, population vulnerability), an exposition risk index for each species was erected. Our results show that about half of all reviewed species, and one third of all species within the EU have an elevated exposition risk, though none of the considered species is categorized Critically Endangered or Endangered under the IUCN Red List, and only two as Vulnerable classified species do. On the other hand, two thirds of the Near Threatened species display an elevated risk. Though variation between pesticide admission zones within the EU is small, differences in the occurrence rates within land use types could be observed. Over all, no group of the studied reptiles is more susceptible than another. To conclude, it is time to consider species-based pesticide risk assessments to improve conservation processes for reptiles and avoid further biodiversity loss.

Key words: Conservation, IUCN Red List, Pesticides, Reptiles
The Balkan Peninsula is part of the globally important Mediterranean basin biodiversity hotspot, where Albania is one of the least known country with a high level of amphibian and reptile diversity. Since distributional records of the local herpetofauna are scarce even for common species and summarized works are outdated, we combined all the available literature and museum records as well as unpublished data to a georeferenced database. Moreover, we have organized several expeditions to Albania since 2009 to explore and collect new herpetofaunistical data. Our database contains nearly 4500 records from all the 14 amphibian and 40 reptile species known from the country. With the help of SEH Grant in Herpetology for 2015, Mapping and Distributions we focused to collect more data from rare or poorly known species and mapping gaps. After contracting the records to 10x10 UTM squares we performed spatial analyses to reveal sampling hotspots and coldspots and visualised diversity maps in GIS. We found that Karaburun, Butrint and Ohrid areas are among the richest ones in herpetological diversity but sampling efforts are frequently biased toward such areas. Valamares and Griba mountain regions are among the least known ones. Also, we revealed priority areas for conservation of the local herpetofauna.

Key words: Balkan Peninsula, biodiversity, biogeography
The Western Ghats of India is one of the mega diversity regions of the world and forms a part of the Western Ghats-Sri Lanka Biodiversity Hotspot. While new species of amphibians are continuously being described from this region, there is limited knowledge on the distribution of several species. Incomplete information on the distribution of species, the so called Wallacean shortfall, is hindering conservation efforts towards this diverse and endemic group of threatened vertebrates. In this study, we investigated distribution of two species of leaping frogs (Anura: Ranixalidae), endemic to northern Western Ghats, viz. *Indirana leithii* and *I. chiravasi*, based on field surveys, genetic identification and niche based modeling. Niche based modeling was performed in DIVA-GIS using 18 bioclimatic variables, enhanced vegetation indices for 12 months and altitude. Environmental parameters for collection points were extracted and principal component analysis (PCA) was performed to understand whether the two species have specific habitat preference. Our analysis suggested that, although the prediction for the extent of occurrence for the two species had some differences, there was no difference in the bioclimatic factors, vegetation and altitude preference by the species and PCA analysis showed complete overlap in the distribution along the environmental variables. However, field observations suggested that there could be microhabitat specificity for each species especially regarding their breeding behavior. For breeding, *Indirana leithii* preferred rock crevices on the cliffs in basalt rocks, covered with algae and water seepage, while *I. chiravasi* preferred crevices in the boulders on rocky outcrops primarily of laterite, laden with mosses and algae. This is the first step towards the prediction of distribution range of these two species and defining their habitat and microhabitat preferences, so as to design and implement conservation action.

**Key words:** Ranixalidae, Wallacean shortfall, Distribution Range, Microhabitat preferences
Preliminary radiotelemetry research of common cobras, *Naja kaouthia* and *Naja siamensis*, in Sakaerat Biosphere Reserve, Thailand

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Common cobras (*Naja* spp.) are responsible for a third of envenomation in Thailand during 1997-2003. Remarkably very few study has promoted snake bite prevention in the past. We conducted research aiming to collect data that will help develop a cobra bite prevention program in Sakaerat Biosphere Reserve, Northeastern Thailand. We captured common cobras from forested and residential areas using active survey, passive trapping and community notification. Eight *Naja siamensis* and twelve *N. kaouthia* were captured from September 2012 to May 2015. Mean adult mass and snout vent length were significantly greater in *N. kaouthia* (1539.2 mm, 1073.6 g) than *N. siamensis* (1177.6 mm, 570.7 g). We fitted two *N. kaouthia* and four *N. siamensis* with internal radio transmitters. Daily radio tracking has begun to elucidate movement patterns for the 6 common cobras tracked for minimum of forty seven days, providing large quantities of preliminary behavioral data (inactive, moving and feeding). By placing camera traps we gained interesting observational data about the snake’s time spent in shelter. We observed human caused mortality, active hunting behavior, predator avoidance and others. Data from behavioral studies, home ranges, and habitat use will be integrated to generate a list of recommendations for inhabitants of rural communities to avoid human snake-bite and improve cohabitation with the snakes.

**Key words:** behavior, conflict, ecology, habitat use, snakes
Comparative skeletal morphology of four species of the genus *Neurergus* (Caudata: Salamandridae)

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We studied the skeletal morphology of *Neurergus crocatus*, *N. kaiseri*, *N. derjugini* and *N. strauchii* and compared their anatomy with each other. Investigation of the skeletal morphology is based on cleared and double-stained specimens collected from Iran and Turkey. According to IUCN (Red List), two species (*N. kaiseri* and *N. derjugini*) of the genus *Neurergus* are Critically Endangered (CR) and others (*N. crocatus* and *N. strauchii*) are Vulnerable (VU). However, very little is known about distribution, abundance and skeletal morphology of these four species. Major differences between the species are as follows: (1) the shape of the parasphenoid, (2) the position of the vomerian teeth and (3) the number of maxillary, premaxillary and vomerian teeth. The shape of the parasphenoid in *N. crocatus* and *N. kaiseri* differs from those of *N. derjugini* and *N. strauchii*. In *N. strauchii* and *N. derjugini*, the vomerian teeth is inverted V-shaped, whereas its posterior ends have a small swelling in *N. crocatus* and *N. kaiseri*. Also, the numbers of teeth are different from one another.

**Key words**: *Neurergus* species, skeletal morphology
Why tortoises tig their food – testing the “tactile tongue” hypothesis

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It has been postulated that extant testudinids (tortoises) use obligatory “lingual prehension” during food uptake. According to this hypothesis, the tongue is the main prehensile organ which ingests the food items from the environment and processes it into the oropharynx. In order to test this statement, we provide a detailed kinematic analysis using digital high-speed video recording systems. Feeding behaviour of nine testudinids belonging to Malacochersus, Testudo, Geochelone, Astrochelys and Manouria was compared with that in three geoemydid species (Cuora amboinensis, C. flavomarginata, and Heosemys grandis). The mode of food uptake significantly differs between the two groups. All geoemyids use jaw prehension when feeding on land. In most tortoises, in contrast, we only detected a contact between the food item and the tip of the tongue prior to jaw closure. The only exception was the ‘basal’, transitional tortoise Manouria emys in which no contact between the tongue and the food was detected at all. We propose that the initial lingual contact with food found in tortoises (except Manouria) is a derived behaviour characteristic only for this group. For tortoises, we were not able to detect a prehensile function of the tongue in our sequences. In most species, the offered food items were grasped in the jaws and not collected or processed by the tongue. We propose that the contact of the tongue with the food prior to food uptake has chemi- (for testing the palatability of the food) or mechano-sensorical (for positioning to the jaws relative to the food during the final head approach prior grasping) function. As the delay between the first detected tongue contact with the food item and the start of jaw closing was very short (less than 150 µs), we propose that the tortoises predominantly use their tongues as a tactile sensor prior to food uptake.

Key words: turtle, feeding, tactile function, coordination, kinematics
How many eggs may a frog spawn during all her life span? A renewable versus a non-renewable stock of oocytes in amphibian ovaries

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Vertebrates display two modes of oocyte recruitment in respect to duration of ability to renovate the pool (stock) of oocytes during the whole lives of females: renolvable (some teleostean fishes) and non-renovable (mammals, birds, reptiles). In amphibians, like in mammals, sexually mature females ovulate several times during their lives. Most anuran amphibians produce high numbers of eggs during several consecutive breeding seasons and therefore it is commonly accepted that oogenesis is cyclic, and the pool of oocytes is renewed after each ovulation by a new wave of proliferation of primary oogonia located in so called germ patches. Formation of a stock of oocytes is a result of two processes: proliferation and depletion by atresia. Our quantitative studies on the grass frog *Rana temporaria* as a model show that primary oogonia in adult female frogs can proliferate, but they fail to differentiate further and do not enter meiosis, and thereby there is no supplementation of new generations of oocytes after each spawning. The final number of oocytes in a female exceeds the number of eggs produced during the potential reproductive life span of this species. Thus, the conclusion is that frog ovaries represent a non-renovable type and produce the complete pool of oocytes once for a life time, like mammals, birds, and reptiles.

Key words: oogenesis, ovary, reproduction
A multilocus dataset for *Lissotriton* newts delimits taxa but fails to recover evolutionary relationships

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Multilocus phylogenetic inference based on NGS datasets may be obscured by high levels of genealogical discordance especially if multiple alleles per locus per OTU are sampled. We sequenced 74 markers (500 bp each, located in 3’ UTR regions) for 128 individuals representative of all subspecies of *Lissotriton vulgaris* and *L. montandoni*. On average, 12.4% (range 2.8-28%) of sites in each sequence alignment was parsimony informative. The delimitation of lineages (in STRUCTURE) was robust and supported by moderate to high exclusive ancestry for each lineage (ensemble genealogical sorting index 0.28-0.69). Apart from *L. montandoni*, some morphologically well-delimited subspecies are also genetically distinct (*L. v. ampelensis*, *L. v. graecus*, *L. v. kosswigi*, *L. v. lantzi*, *L. v. meridionalis*, *L. v. schmidtlerorum*), however *L. v. vulgaris* is composed of southern (southwest of the Carpathians) and northern (north and east of the Carpathians) lineages, the latter forming a clade with *L. v. meridionalis*, making the nominative subspecies paraphyletic. Moreover, *L. v. ampelensis* included some individuals with *L. v. vulgaris* morphology. Well-supported phylogenetic relationships included the basal placement of *L. montandoni*, and *L. v. kosswigi* and *L. v. graecus* as a sister clade to all other *L. vulgaris* lineages. The relationships among the remaining lineages were inconsistent in phylogenies based on the multilocus coalescent (*BEAST), Bayesian concordance analysis (BUCKy) and a supermatrix approach (MrBayes) despite the implementation of subsampling strategies aimed at reducing the complexity of the dataset, and by treating individual alleles as OTUs. We hypothesize that (i) retention of ancestral alleles due to large effective population sizes and (ii) gene flow between some of the newt lineages, including non-sister lineages, may have overwhelmed the phylogenetic signal in the dataset. We assess the extent to which these factors have contributed to the inconsistencies in our results using approximate Bayesian computation modeling.

**Key words:** *Lissotriton*, NGS, multilocus, phylogeography, species delimitation
Preliminary Molecular Clock Analysis of Western Ghats Endemic Genus Indirana

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Genetic separation of species belonging to the Western Ghats endemic genus Indirana, was studied using molecular clock analysis. Separation of Ranixalidae and Micrixalidae dates back to late Cretaceous (approx. 90 MYA). However the speciation within Ranixalidae appears much later. The first one to separate was Indirana leptodactyla about 60 mya, as a basal clade within the family Ranixalidae. Indirana leithii, now known to be restricted to the Northern Western Ghats, appears to be separated from the other members of genus Indirana around 32 MYA. However, Indirana diplosticta, a member of leithii group forms a monophyletic clade with Indirana semipalmata, a member of beddomii group. This clade appears to be separated from remaining members of beddomii group at around 21 MYA. This interesting monophyletic association of species of two different groups is perhaps due to the secondary expression of ancestral characters in Indirana diplosticta (such as – shorter first finger and smaller tympanum), which leads to its taxonomic inclusion in the leithii group. Indirana gundia separated from the other two species viz. Indirana chiravasi and Indirana sp. at around 14 to 15 MYA. Indirana chiravasi and Indirana sp., from Northern Western Ghats, were earlier considered to belong to the same species complex due to their morphological resemblance with Indirana beddomii. With current analysis, it is evident that these two species have been separated from each other at around 10 to 12 MYA. This is however a preliminary analysis as we could not incorporate the remaining species Viz. Indirana beddomii, Indirana brachitarsus, Indirana longicrus and Indirana tenuilingua due to their unresolved taxonomic status. Further, the DNA sequences for Indirana phrynoderma are not available in the GenBank. Inclusion of these species in the analysis may reveal more comprehensive picture with respect to the Vicariance of the Genus Indirana after the Deccan Trap formation.

Key words: Vicariance, Western Ghats, Endemic, Ranixalidae
Saline bomb crater ponds as amphibian habitats on the Great Hungarian
Plain

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Salinity is an important factor for population declines, distribution and diversity
patterns in amphibians. Amphibians usually inhabit freshwater environments but
some species are adapted to wide salinity gradients. A number of studies were
performed on different species to reveal their tolerance to salinity, but there is only
a limited empirical evidence on its community structuring role. Thousands of
saline bomb crater ponds on the Pannonian Plain represent secondary habitats. We
conducted our study on a sodic grassland, holding more than 100 such crater ponds
within 1 km². We aimed to assess the relative contribution of local variables and
spatial factors on the assembly of the amphibian communities. Hand netting
supplemented by visual searching was used to record amphibians and dip netting
for frog larvae and newts. Conductivity, pH, water temperature, water depth and
vegetation cover were determined on the field. The ponds exhibited a wide range
of salinities along with a wide range of vegetation cover. We detected 7 amphibian
taxa in the study area and 5 of them (Bombina bombina, Hyla arborea, Pelobates
fuscus, Pelophylax esculentus complex, Triturus dobrogicus) were successfully
breeding in 2014. The fire-bellied toad (Bombina bombina) was the most abundant
species, which occurred in almost all of these ponds. Despite their anthropogenic
origin, we found that these ponds served as important habitats for amphibians and
should be maintained rather than eliminated.

Key words: Amphibia, Bombina bombina, salinity, pond, community
Insufficient efficacy of the Natura 2000 network in Bulgaria to protect suitable habitats of target amphibians and reptiles

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Bulgaria is amongst the countries with the highest percentage of area protected through its national ecological network Natura 2000, with SCIs (Habitats Directive, 92/43/ECC) covering 30.5% of the country’s territory in 2012. Thirteen species of amphibians and reptiles occur in Bulgaria, subject to Annex 2 of the Habitats Directive: four newts, two toads, four turtles and tortoises, and three snakes. To evaluate the efficacy of Natura 2000 to protect the target reptile and amphibian species at the national level, we modeled and compared the areas of the suitable habitats within and outside of the national ecological network. For each species, we ran spatial distribution models with program Maxent, using real observations. We used available layers with climatic and geographic variables, and actual land use. The resultant potential distribution maps were reclassified in four suitability classes: class #1, unsuitable (Maxent values 0–0.1); #2, marginally suitable (0.11–0.33); #3, moderately suitable (0.34–0.66); #4, optimal (0.67–1). The results show that the percent of suitable territories outside of the network increased directly proportionally to the degree of suitability of the habitats for each species (class #2 = mean 37.95% ± 13.01; #3 = 47.15% ± 12.99; #4 = 59.65% ± 11.38). Evidently, the majority of class #4 (optimal habitats) territories are not protected though the Natura 2000 network. Amongst the species with the highest percent of moderately suitable and optimal habitats (classes 3 and 4) outside of the network are Mauremys rivulata (74%, 82% respectively) and Elaphe quatuorlineata (57%, 78%). A major problem we identified with the network is that in its design phase the territories were not tested for connectivity. When creating such ecological network and defining its borders, the connectivity between its protected sites must be taken into consideration, through collecting sufficient preliminary data on the distribution of the target species.

Key words: conservation, legislation, Habitats Directive, connectivity
The evolution of sexual dimorphism in salamandrids: patterns and processes

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Particular the salamanders of the family Salamandridae (Amphibia: Caudata) are suitable for studying inter- and intraspecific patterns of sexual size dimorphism (SSD) because they exhibit an extraordinary diversity of reproductive modes including oviparity and viviparity combined with variable morphologies and body shapes. A diverse array of species representing different mating systems, reproductive modes and life histories have been analysed morphometrically. We have also followed the ontogeny of sexual dimorphic traits by including age data and finally we have integrated the most recent phylogenetic hypothesis to follow the evolution of particular morphological traits. Our comparative multidimensional analysis aids in understanding the scenario behind the evolution of sexual dimorphism in a phylogenetically ancient vertebrate group.

\textbf{Key words:} Salamandridae, life history, sex, mating
Status of molecular exploration of global amphibian diversity

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Herein, we assess the historical dimension of our knowledge on global amphibian species diversity and illustrate how our recognition of species diversity has changed through more than 250 years of discoveries based on range estimates of 7,038 species (95.8\% of all amphibian taxa). Global coverage of amphibian diversity is estimated based on the twelve most common molecular markers, including 174,371 sequences. We hypothesize that (i) integrative taxonomy utilizing genetic information has accelerated the recognition of endemic, often cryptic taxa and ultimately led to a boost in discoveries of species. While the delimitation of early-described species relied on morphological (and ecological) characters, molecular data potentially allows a much finer distinction between taxa, which ultimately leads to a boost in species descriptions and a higher percentage of identified cryptic, often endemic taxa as new techniques become available. If so, the average minimum genetic distance between newly recognized species and the known set of species at a given time step will decrease. However, (ii) as the availability of molecular methods is not equally distributed around the globe, this translates in an uneven temporal and spatial detection rate. While marker selection predominantly depends on applicability for certain systematic studies, preferences for established genetic markers also result in an uneven global coverage causing a spatial bias in the coverage of the local amphibian fauna affecting the likelihood to detect new species via molecular methods. At the same time large scale phylogenies may suffer from this spatial bias as specific groups are underrepresented.

Key words: global amphibian species diversity, species discoveries, molecular markers, spatial bias
Life-history correlates of evolutionary shifts along the oviparity-viviparity continuum in lacertid lizards

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Most live-bearing squamates exhibit a primitive (lecitotrophic) viviparity which can be described as a pronounced retention of eggs in the oviducts with accompanying shifts in some other quantitative traits. Viviparity of this kind may thus be viewed as an endpoint along an oviparity-viviparity continuum (OVC). Theory predicts that a transition from oviparity to viviparity is associated with evolutionary shifts in multiple life-history traits. These predictions have mostly been tested via broad comparisons of viviparous vs. oviparous species, whereas life-history consequences of smaller shifts along the OVC, which occur within the egg-laying reproductive mode, remain poorly studied.

Lizards of the West Palaeoarctic tribe Lacertini present a promising model for studying initial stages towards viviparity. They show a marked variation along the OVC among and within species. My talk will confront the patterns of intraspecific life-history divergence in two widespread lacertid lizards, Zootoca vivipara (which includes both oviparous and viviparous lineages) and Lacerta agilis (Roitberg et al., 2013, 2015, see www.eroit.allrital.de for details), with the patterns of interspecific variation (Braña et al., 1991; Braña, 1996). A pronounced parallelism of life-history variation along the OVC within and among species was found. The most consistent pattern is a decrease in mean offspring size in species or intraspecific lineages with longer vs. shorter egg retention. This trend is apparently accompanied with increasing the total clutch mass relative to female mass, and increasing female body size relative to male body size. The revealed patterns will be discussed in the context of several models of life-history evolution, such as Darwin's fecundity-advantage hypothesis and the Winkler & Wallin model.

This study is part of a project supported by the Deutsche Forschungsgemeinschaft (grant RO 4168/1).

Key words: Lacerta agilis, life-history, lacertid lizards, oviparity-viviparity continuum, Zootoca vivipara
The Lebanon Mountain Viper, *Montivipera bornmuelleri* is the only snake species present in the Aayoûn es Sîmâne plateau in the Sannine Mountain in Lebanon. This study was conducted at a location of around 2000m in altitude in that region. Various sporadic field observations about the snake’s habitats and microhabitats have been made since 1992. More recently, thirteen specimens were radio-tracked from June till October using implanted VHF micro-transmitters: 5 individuals in 2013 and 8 individuals in 2014. General observations and radio-tracking data revealed a fairly consistent pattern of annual activity in most individuals: they hibernate on hilltops, which are mostly rocky and barren, emerge from hibernation and spend a few weeks on high ground then, during June, they migrate downhill to adjacent lush vegetation covered depressions where they spend the summer. During their stay in these depressions, their movements are mostly restricted to within several meters, but a few individuals have shown much wider movements. In September-early October, they migrate back to hilltops where they hibernate. These observations are concordant with the pattern of snow cover in the study area. The hilltops are the first spots to become exposed during the spring snowmelt in this area that remains totally snow-covered from late November-December till March-April (varying from year to year). These snakes, thus, would have an early start for their (mostly breeding) activity.

**Key words:** Lebanon, vipers, ecology, *Montivipera bornmuelleri*, hibernation, home range
Effects of dehydration and salt loading on the osmoregulatory capacities in Moroccan spiny-tailed lizard, *Uromastyx nigriventris* (Reptilia: Agamidae)

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Animals inhabiting arid environments use a variety of behavioural and physiological strategies to balance water and salt budgets. We studied the effects of dehydration and salt loading on osmoregulatory capacities in a large herbivorous desert lizard, the Moroccan Spiny-tailed lizard *Uromastyx nigriventris*. These lizards select plants with a high K to Na ratio of 15 to 20, and like other herbivorous lizards, effectively eliminate the extra electrolytes load, mainly via pair active nasal salt glands which exude the extra ions from blood plasma. Here we present results of a series of laboratory experiments which tested a five-week food and water deprivation and the excretory response of nasal salt glands, during a short period of five days, following salt loading by daily injections treatment of KCl, NaCl, potassium acetate and sodium acetate. During food-water deprivation, dehydrated lizards lost 32% of their initial body mass (with a decrease of the Body Condition Index from 1.49 to 1.07 g/cm) and 46% of the tail volume as an index of energy (fat) storage. Plasma osmolality significantly increased by 20%. There were also significant increased plasma sodium (hypernatraemia), chloride and total protein concentrations showed. On the other hand, there was no significant decrease in the plasma glucose level. Most of the salt loaded lizards secreted far more K$^+$ than Na$^+$ via the nasal glands, even after NaCl loading. The K$^+$/Na$^+$ ratio decreased only after two to three repetitive NaCl injections but insufficient Na was eliminated. Two successive KCl injections were successfully eliminated but daily natural average K$^+$ administration induced progressive hyperkalemia. These experimental data agreed with previous observations showing variations of plasma Na$^+$ and K$^+$ concentrations in free-living lizards. The nasal gland constitutes the main route of Cl$^-$ excretion but the Cl$^-$(Na$^+$ + K$^+$) ratio may vary according to observations in other herbivorous species.

**Key words:** *Uromastyx*, dehydration, salt loading, nasal gland, electrolytes, osmoregulation
Influence of North Atlantic Oscillation (NAO) climatic pattern on Speleomantes strinatii population dynamics

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The North Atlantic Oscillation (NAO) climatic pattern regulates the global atmospheric circulation in the northern Hemisphere. The NAO index has been often correlated with vegetation growth, plant phenology and animal population variability in northern Europe and in the western part of the Mediterranean. However, in the Mediterranean, the influence of the NAO has rarely been analysed in relation to the long-term population dynamics of amphibians. In this study, the relationship between different seasonal NAO indexes (i.e. spring, summer and winter) and the population variability of the terrestrial European plethodontid Speleomantes strinatii has been examined. The focal salamander population was monitored inside an artificial cave in NW Italy, for 24 consecutive years, this time period corresponding to about two salamander generations. A population index, validated by absolute abundance estimation obtained through temporary removal sampling, was used to describe the salamander dynamics. The relationships between population fluctuations and spring, summer and winter NAO indexes were assessed by cross-correlation function (CCF) analysis, after prewhitening the time series by autoregressive moving average (ARMA) statistical modelling. There was no significant relationship between the salamander population time series and the spring and summer NAO indexes. However, the salamander dynamics appeared negatively correlated to the one-year ahead NAO index during wintertime (cross-correlation = 0.621, P = 0.018). In southern Europe, positive winter NAO values generally correspond to dry and cold weather conditions. These findings strengthen the results from previous studies that suggested the high sensitivity of temperate amphibian populations to unsuitable winter climatic conditions.

Key words: Amphibians, Plethodontidae, salamanders, cross-correlation, NAO, climate
Diet and stable isotopes uncover trophic-niche divergence and ecological diversification processes for endemic reptiles in Socotra

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Ecological diversification on islands typically results in divergence of ecological niches. As diet is a major component of species niches, we hypothesize that sister species within island monophyletic groups diversify in their dietary preferences. We have examined this hypothesis with six endemic reptile species from Socotra Island (Yemen). Trophic niches were examined by the analysis of faecal samples and carbon and nitrogen stable isotopes. We collected pellets and tail tips (for isotopes) during two visits in 2013 and 2014 to Socotra. Specific trophic niche widths inferred from stable isotopes were estimated from ellipse-based metrics, and interspecific differences were compared by linear mixed models using lithology, altitude and microhabitat of sites where animals were collected as environmental predictors. We found similar interspecific divergence using faecal samples and isotopes. For both isotopes, models detected interspecific differences between sister species (i.e. trophic niche divergence) and also diet similarity between species of distant lineages (i.e. ecological convergence). In a phylogenetic context, trophic-niche interspecific comparisons highlight the mechanisms (microhabitat, altitudinal and body-size segregation) that are driving ecological diversification and speciation in Socotra.

Key words: Ecological speciation, endemism, reptile, Socotra, stable isotopes, trophic niche
Taxonomic and functional responses of reptiles to fire

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Fire is a common disturbance in many regions of the world, and a fundamental element to understand ecosystem functioning and structure. Fire simplifies the habitat structure and increases open areas. In these environments we expect that some Mediterranean reptiles would be favoured in early post-fire stages, being replaced along the post-fire succession by other species adapted to un burnt mature habitats. Changes in fire regime (e.g. fire frequency and extension) can modify this dynamic specially threaten species that need mature un burnt habitats to persist. During the last eight years, I have analyzed responses of reptile assemblages to fire in eight localities across the Western Mediterranean. I registered 4000 records of 29 species along 250 transects. In general, the number of reptiles increases in burnt areas although the species richness decreases. However, I found opposing responses to fire according to the biogeographic affinity and particular physiological, reproductive and microhabitat traits of species. I also found that some threatened species are severely impacted by fire. My results confirm that the response of Mediterranean reptiles to fire is complex and diverse. Consequently, fire management in terms of biodiversity conservation have to take into account which species are or not favoured by fire. The final objective is to translate my results to stakeholders and conservationists to design adequate guidelines to protect species from fire, especially under a future scenario of increasing fire frequency and extension.

Key words: reptile conservation, fire, Mediterranean basin, functional response
Amphibians as indicators of favourable Mediterranean temporary ponds (SW Portugal)

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The coastal plain of southwest Portugal is classified under the European Natura 2000 Network as Site of Community Importance (SCI Costa Sudoeste), because it also hosts a large number of priority Mediterranean temporary ponds (TMP). In the last two-decades, modern industrialized agriculture and tourism are causing a steep decline of this European habitat (H3170*). TMP are very shallow temporary ponds (less than 0.5m deep) which fill in during Autumn and Winter and dry out in late spring. From January 2014 until May 2015, about 82 TMP of this SCI were assessed for their conservation status, based on strictly plant criteria (H3170*). This assessment revealed 21 TMP in favourable condition, 25 in inadequate and 36 in unfavourable condition. At same time the amphibian richness was surveyed for this TPM set. Regionally 13 amphibian species may be found, but on average we found 3.38±2.32 species per pond, with a maximum of 8 species. The five most recorded species were Pelobates cultripes (68.3% of all ponds), Pleurodeles waltl (47.6%), Hyla meridionalis (45.1%), Pelodytes punctatus* (40.2%) and Triturus pygmaeus (34.2%), while the less one was Salamandra salamandra (2.4%). An indicator species analysis was run to check if any amphibian species are associated with the TMP conservation status refereed above. For favourable TMP, Triturus pygmaeus (IV = 0.74) and Pleurodeles waltl (IV = 0.72) show a high indicator value (IV) with p-value <0.001 based on 2000 randomizations (Monte Carlo test), followed by Pelobates cultripes (IV = 0.61), Pelodytes punctatus* (IV = 0.58) and Lissotriton boscai (IV = 0.50) with p-value <0.05. There were no significant species associations neither with inadequate nor with unfavourable TMP conservation status. These results are relevant to implement a quick TMP assessment for technical staff.

Key words: amphibians, Mediterranean temporary ponds, conservation status, Portugal
Home ranges of parthenogenetic and bisexual species in a community of Darevskia (Italic) lizards

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We analysed the home ranges of a community of Darevskia rock lizards composed of a bisexual species (D. valentini), two parthenogens (D. armeniaca and D. unisexualis), and two backcross forms between bisexual and unisexual forms. We estimated home range (HR) areas of ink-marked, GPS-locate lizards using Minimum Complex Polygon (MCP) and 95% of the locations for those individuals with five or more sightings. We compared home range size, perimeter, and total travelled distance between species accounting for lizards' morphology. We also counted the number of individual HRs intersecting other individual HRs and those with no overlap and measured the overlap proportion among MCP intersections. Of the 32 individuals captured we were able to calculate home ranges for 17 individuals with 123 records. The bisexual D. valentini was the species with the largest home ranges, travelled distances, and the most intersections. No differences between unisexual species and backcrosses were recorded for any comparison. In males, HR size and perimeter were related morphological characteristics. Contrarily to what has been described in allopatry, we found unisexual species with smaller HR and less overlaps than sympatric bisexual species. We tentatively suggest that the presence of potential bisexual partners might increase sexual competition among parthenogenetic females while differences in habitat use should also be considered.

Key words: reptiles, parthenogenesis, Armenia, Geographical Information Systems, spatial statistics, minimum convex polygon
Winter survival of juvenile toads (*Epidalea calamita, Bufotes viridis*) is the principal driver of population dynamics

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The size of amphibian populations varies considerably between years, so that systematic trends in dynamics are difficult to detect. Informed conservation management of presumably declining populations requires the identification of the most sensitive life stage. In temperate-zone anurans there is growing evidence that juveniles hibernating for the first time suffer from substantial winter losses. In two syntopic toad populations (*E. calamita, B. viridis*) we monitored survival of such juveniles during four consecutive winters in the natural habitat and during experimental hibernations in temperature-controlled chambers (3-20°C, natural light-dark cycle). Specifically, we test the hypotheses that (1) winter mortality of first-hibernating juveniles is the principal driver of population dynamics, and that (2) mortality rates differ between the two species. Parameters quantified were size-dependent winter mortality and body condition of pre- and post-hibernating juveniles. Field data provided evidence for the dominant role of winter mortality of first-hibernators in population dynamics, but individuals of all size-classes were able to cover metabolic costs with pre-wintering energy storage and occasional food uptake. Choice of hibernacula differed in *E. calamita* between small- and medium-sized individuals and also between the two species suggesting distinct mortality risks. The inability of small-sized *E. calamita* to reach frost-proof hibernacula by burrowing, and the exposure of small-sized *B. viridis* to predators are the most probable causes of size-assortative winter mortality. *E. calamita* juveniles will probably benefit from rising average winter temperatures in future by decreased risk of freezing to death. Predator-caused winter mortality of *B. viridis* juveniles, in contrast, will probably not be affected by climate warming.

**Key words:** Amphibians, size-assortative winter mortality, hibernacula, predator, condition
Do displaced newts (*Triturus cristatus, Lissotriton vulgaris*) navigate?

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Initial orientation of crested newts *Triturus cristatus* and smooth newts *Lissotriton vulgaris* was measured following passive displacement to arenas placed at 12 test sites 0.213–24.2 km distant from the breeding pond. Newts were transported by car without visual and olfactory outward journey information, but full access to local orientation clues at the release site during the night and early morning before testing. Within the area of previous migratory experience, the initial directional choice of both species was significantly directed to home. Outside the area of familiarity, the initial orientation was indistinguishable from random in all but one site each at 3 and 20 km distance, respectively. We suggest that short-distance orientation was probably based on olfactory beaconing and on a learned spatial map integrating visual and olfactory information. The failure of homeward orientation following long-distance displacement indicates the absence of a spatial map which allows for extrapolation into unknown areas. The geomagnetic features of the study area include regional-scale irregularities probably impeding to derive reliable information from a geomagnetic map. In conclusion, newts seem to be unable to navigate.

**Key words:** orientation, navigation, map, long-distance homing, passive displacement, arena
To the problem of aging of small arid lizards

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Study of the growth layers in recording structures including tubular bones is a useful tool for age determination in animals and for analysis of their growth rates. It is connected with the expansion of marrow cavity, which results from endosteal resorption as the bone grows in thickness. As a result some of the growth marks, which were deposited first, can be partly or even completely removed. The resorption process usually stops after maturation (Castanet, Smirina, 1990). The goal of our study was to test the hypothesis of this arid lizards “ephemerity” using skeletochronological method. It was supposed that lizards born in June–July, maturated to the spring of the next year and died after the realization of reproductive function (Bogdanov, 1960, 1962; Shammakov, 1981). We examined 50 individuals of one of the smallest arid agamids Phrynocephalus interscapularis (Lichtenstein, 1856) collected in frame of an joint Australian Research Council project in Uzbekistan in 2003 (Cleemann et al., 2008) and stored in Zoological Institute, Russian Academy of Sciences.

Individual age was determined by counting the number of growth layers (Lag-s) formed in the humerus (Castanet, Smirina, 1990). Cross sections (18-20 mkm thick) from the middle of diaphysis of humerus were prepared using freezing microtome and stained with Ehrlich hematoxylin embedded in glycerol and examined under a light microscope at a magnification 10 x 10 or approximately 10 X 40. Preliminarily the bones were decalcified in 4-5% solution of nitric acid. Our results show the maximal age of lizards up to three years. This result coincides with the previously earlier results of capture-recapture method (Kulikov, Semenov, 1984). The study was supported by RFBR 15-04-01730 and by participation of Zoological Institute (№01201351184).

Key words: aging, Agamidae, Phrynocephalus interscapularis, skeletochronology
Tolerance to salinity and dehydration in the Sahara Desert’s Blue-eyed Turtle, *Mauremys leprosa saharica* Fritz, 2006 (Testudines: Geoemydidae) from an isolated brackish pond in the Lower Draa basin, Southern Morocco

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The peripheral populations of the Blue-eyed Pond Turtle, *Mauremys leprosa saharica*, in the southernmost species distribution range in the Lower Draa, Southern Morocco, are exposed to extreme environmental conditions of arid climate and anthropogenic and climate change mediated water and land salinisation. In the present study, we investigated a small and isolated population of this turtle at Sidi El Mehdaoui oasis in the Lower Draa, in order to assess its osmoregulatory capacities and tolerance to salinity and dehydration. Upon *in-situ* captures, turtles were weighed and measured for shell morphometric characters; blood and voided urine samples were taken. Tests of exposure to salt water and maintenance out of water (aestivation simulation) were carried out. Osmolalities and osmolytes (Na\(^+\), Cl\(^-\), K\(^+\), urea and glucose concentrations) in plasma and urine were determined. The turtles are not able to survive in brackish waters with salinity over 24% seawater; they depend on their water stored in the bladder which allows them to osmoregulate and ionoregulate till the isoosmocity level beyond which osmotic and ionic anhomeostasy (hyperosmolality, hypernatraemia, higher plasma urea and chloride levels) occurs. Osmoregulatory and ionoregulatory in the Blue-eyed Pond turtles are low and limited and do not allow them to survive for long term periods in salt water or out of water because of dehydration indicated by progressive weight loss to a critical threshold. The increased drought and water and land salinisation related to anthropogenic activities and climate change represent great threats to the long term persistence of the vulnerable marginal populations of the Saharan Pond turtle and their habitats. So conservation measures of these populations are particularly urgent.

**Key words:** *Mauremys leprosa*, Draa watershed, salinity, dehydration, osmoregulation, conservation
Genetics of Dutch Wall Lizards (*Podarcis muralis*); on the vitality of the only native population and the threat of introduced populations

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The wall lizard (*Podarcis muralis*) is a very rare species in the Netherlands, with only one native population. This population was near extinction in the eighties of last century. A study based on nuclear DNA (microsatellites) was conducted to look for signs of inbreeding, a genetic bottleneck, effective population size and possible genetic isolation of sub-populations (e.g. the functioning of the meta-population). We found that the Dutch population shows signs of inbreeding. A busy road causes genetic isolation of sub-populations and prevents the optimal functioning of the meta-population. Additionally we found that the Dutch wall lizard population is genetically different from nearby German populations, and appears to have a lower effective population size. Measures are proposed to guarantee the long-term survival of the species in the Netherlands.

A second supposed threat to our native wall lizards are the presence of introduced wall lizards, through hybridization and competition over resources. The number of reported introductions has been growing fast over the past 15 years. Based on mitochondrial DNA, we looked at the origin of ten introduced, self-sustaining populations of wall lizard. These populations appeared to originate from six different clades, and often haplotypes from more than one clade were present at a single location, indicating multiple introductions. Haplotypes from the Eastern France clade (that also includes the Netherlands), were encountered most often. Within our only native population a few suspect individuals with an exotic haplotype are present, but there is no sign yet for a take-over by non-native wall lizards. The threats of introduced wall lizards and possible actions are discussed.

**Key words:** *Podarcis muralis*, genetic vitality, inbreeding, isolation, introduction, non-native
Is the amphibian pet trade a vector for *Batrachochytrium salamandrivorans* in the Netherlands?

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*Batrachochytrium salamandrivorans* [Bsal] is a newly discovered chytrid fungus which causes massive die-offs of Fire salamanders (*Salamandra salamandra*) in the Netherlands and Belgium. The fungus poses a significant threat for salamanders and newts, and herewith also for the ecosystems they live in. The global trade in exotic animals may facilitate the spread of diseases, and it seems plausible that the international trade in amphibians has unintentionally aided in the distribution of Bsal. The Netherlands Ministry of Economic Affairs has asked RAVON to conduct a study on the possible pathways of Bsal into the Netherlands, with emphasis on the role of amphibian trade. In this study we focussed on the role of imported amphibians. We investigated possible pathways (both introduction and dispersal routes) within the Netherlands, ranging from whole sale pet traders to private keepers. We will present the results of our research and discuss the encountered challenges as well as the necessary prospective steps to be taken.

**Key words:** amphibians, disease, pet trade, *Batrachochytrium salamandrivorans*, pathway
The uncertain future of the Dutch fire salamander population infected with *Batrachochytrium salamandrivorans*

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Amphibian biodiversity loss is emblematic of the current global biodiversity crisis. One of the causative agents of this is amphibian chytridiomycosis, which is called to be the worst infectious disease ever in terms of the number of species impacted and its propensity to drive them to extinction. Chytridiomycosis is caused by two fungi: *Batrachochytrium dendrobatidis* and *B. salamandrivorans*. Both fungal species share some characteristics: they both induce a lethal skin disease, severe population decline and both are capable of infecting a broad amphibian host range. However, host responses to infection with *B. dendrobatidis* are geographically and taxonomically inconsistent and are influenced by environmental factors. Since *B. salamandrivorans* and *B. dendrobatidis* both occupy different ecological niches, these both pathogens may – their spread being enhanced by the global trade and traffic – cause the total collapse of amphibian populations, and through this disturb complete ecosystem stability. Amphibians play, after all, a central role in natural systems, both as predator and prey. In this presentation we will briefly describe the collapse of the fire salamander (*Salamandra salamandra*) population in the Netherlands due to *B. salamandrivorans*, and we will present the current state of this population. Additionally we will discuss the status of newts in the vicinity of this outbreak site. We will also discuss the role of soil in the epidemiology of *B. salamandrivorans*, and research on the possible pathways of this fungus.

**Key words:** Chytridiomycosis, Caudates, The Netherlands, *Batrachochytrium salamandrivorans*, Population dynamics, Threatened species
Detection of olm (*Proteus anguinus*) environmental DNA in karst groundwater

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The olm (*Proteus anguinus*), is the only cave-dwelling vertebrate of Europe. It inhabits subterranean karst waters of the Dinaric Karst in the North-western Balkan Peninsula. Since the beginning of the research on the olm, the basic but particularly important question of its exact distribution has been difficult to address. Due to inaccessible subterranean habitat its presence can only rarely be confirmed by classical survey methods such as trapping and visual encounters. To overcome this problem, we employed environmental DNA (eDNA) method using a SYBR chemistry-based real-time PCR assay with two sets of specific primers to amplify short mitochondrial DNA sequences in the 16S rDNA gene (153 bp) and in the control region (106 bp). The specificity of the assay was previously tested on trout, crested newt and human DNA. In controlled conditions at the Tular Cave Laboratory the minimum density at which its DNA could still be detected corresponded to one animal per 256 m$^3$ of standing water, when sampling 20 L of water. The method, tested at three Slovenian field test sites occupied by different lineages of the olm (the springs of Vir pri Stični and Mahniči, and the cave Kompoljska jama), was 100% effective. Subsequently, a pilot survey of its distribution was conducted along the southern limit of its known range in Herzegovina and Montenegro. Using DNA-based identification we unequivocally established the presence of *Proteus* at four sites, and found its likely traces at additional eight sites – all new localities for this species. With real-time PCR-based method of eDNA detection, we developed an innovative non-invasive approach for reliable and efficient monitoring of this species and possibly for other aquatic cave-dwelling organisms.

**Key words:** environmental DNA, real-time PCR, distribution, non-invasive sampling, *Proteus anguinus*
Anuran hybridization and sex chromosome evolution in space and time

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Reproductive isolation (RI) is expected to accumulate with divergence time but the time taken to speciate may vary between organisms. In anurans, laboratory crosses can still produce viable hybrids >20 My after separation but speciation speed in closely related lineages under natural conditions is poorly studied. Heteromorphic sex chromosomes (SC; occurring e.g. in mammals, birds) have key roles in driving RI. However, most amphibians exhibit indistinguishable (homomorphic) SC, whose evolutionary significance is not well known. Phylogeographic analyses of Palearctic tree frogs (Hyla arborea group) and green toads (Bufo viridis subgroup) allowed identification and dating of secondary contacts. In four tree frog species and all diploid green toad species, we established homologous, homomorphic, occasionally recombining XY-SC. Using transcriptome-based microsatellites, we study introgression in hybrid zones of lineages that diverged at different times. Introgression at autosomal loci should be greater than that at SC loci, as expected from the Haldane’s rule, stating that in interspecies crosses the heterogametic sex (XY-♂♂ or ZW-♀♀) suffers greater fitness loss than the homogametic sex (XX-♀♀ or ZZ-♂♂). Indeed, genome-wide vs. linkage-group-specific hybrid index in a tree frog hybrid zone (Hyla arborea, H. orientalis, divergence time 5 My showing moderate RI) revealed larger autosomal than sex-linked introgression. However, in two green toad hybrid zones we observe either no difference between both marker types (B. balearicus/B. siculus, diverged 2.7 My; strong RI) or even more sex-linked than autosomal introgression (B. balearicus/B. viridis, divergence 1.9 My; weak RI). The latter may points to unknown evolutionary features of homomorphic SC in early speciation. If confirmed, our findings might have implications on the recognized anuran biodiversity.

Key words: Amphibia, Hylidae, Bufonidae, speciation, hybrid zone, introgression, sex chromosomes, evolution
Mito-nuclear analysis of northern rain frogs (Anura: Craugastoridae) reveals taxonomic–genomic incongruence

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Direct-developing northern rain frogs (Craugastor bocourti Species Series) occur in Mexico and northern Central America. There are 19 species in the group making it the most species rich northern Craugastor lineage. However, all of these taxa were described on the basis of subtle morphological differences and have never been examined using molecular data. We used a multilocus dataset (one mitochondrial [mtDNA] and four nuclear [nDNA] gene fragments; totaling 3048 base pairs) to investigate phylogenetic relationships among 57 frogs referable to 12 species with a particular focus on species from Guatemala. Using Maximum Likelihood and Bayesian criteria to infer phylogenies on separate mtDNA and nDNA datasets, we identified several matched clusters of individuals. Matched mtDNA/nDNA clusters were often incongruent with morphology-based taxonomy. For example, two shallow haplogroups contained individuals referable to at least five species. These findings may indicate that in certain lineages species richness has been overestimated. While our gene tree analyses provided statistical support for the monophyly of multiple species within the C. bocourti Species Series, interspecific relationships were mostly unresolved. Coalescent species tree analysis recovered a concordant pattern with few resolved interspecific relationships. Comparisons with data from previous studies that reported better-resolved relationships among Craugastor species revealed that the mitochondrial and nuclear genes used in this study are relatively conserved. We suspect this characteristic of our dataset explains the poorly resolved interspecific relationships we observed across phylogenetic analyses.

Key words: Amphibian, Hylactophryne, Terrarana, Honduras, *BEAST, Anura
Reinventing the image of king cobra, *Ophiophagus hannah*, as a flagship species in rural Thailand

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King cobra, *Ophiophagus hannah*, the largest venomous snake in the world, fits much of the criteria for a flagship species due to its high profile, large home range, and its Vulnerable status under the IUCN Red List; however, the major flagship criteria that king cobra does not meet is a *positive* name recognition. Since 2011, we have conducted king cobra based community outreach and student education programs to generate positive public awareness for king cobra at Sakaerat Biosphere Reserve in rural Northeast Thailand. We conducted a king cobra awareness campaign among local stakeholders by distributing informational flyers and providing a free snake removal service, with the next step, opening a booth in local markets every week. Through radio-telemetry field work we performed 479 outreach interactions with locals. Moreover, we held two workshops that allowed villagers to interact with snakes and learn about king cobras during surgical preparation. Our work has changed perception of many locals and led to the rescue of 8 king cobras, which would have otherwise been killed by villagers. For 3 years, we also educated 5,332 students of 58 schools from across Thailand on snake ecology and conservation. At first, most of students have negative perception towards king cobras and snakes in general. However, after we allow them to interact with nonvenomous snakes in our program, many students including their teachers changed their minds. They have less fear and understand that snakes intentionally avoid people. Student surveys revealed the king cobra as the most feared snake but the local hospital records for the past 5 years and published national surveys show no king cobra bites at all. These results show the major gap between perception and reality, and the potential for altering how people view king cobras since king cobras rarely have negative interactions with people.

**Key words:** community outreach, conservation, education, public awareness, Sakaerat Biosphere Reserve, snakes
The frequency of degenerating germ cells in the ovaries of water frogs

*Pelophylax esculentus* – complex

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*Pelophylax esculentus* is the fertile hybrid of *P. ridibundus* and *P. lessonae*. During gametogenesis one of the parental genomes is removed from the germ line cells, whereas the second one is clonally transmitted to the gametes. The development of ovaries is divided into ten stages. In hybrids, the maturation of the gonads is delayed in comparison to the parental species. This may result from complex processes of rejecting the genome, potentially responsible for increased cell abnormalities of germ cells. The aim of the present study was to assess the frequency of degenerating germ cells in the ovaries. Ovaries of seventeen individuals were classified as stages III–VI (undifferentiated gonad-first diplotene oocytes). Degenerating germ cells with positive expression of active caspase-3 were detected in tissue sections using immunohistochemical method. Gonadal volume and number of degenerating cells were estimated for each gonad. For statistical analysis, the Mann-Whitney U-test and Spearman’s correlation test were used. Histological analysis demonstrated that in the ovaries of *P. lessonae* and *P. ridibundus* (n=6) diplotene oocytes were more prevailing than in *P. esculentus* (n=11). The number of degenerating germ cells was significantly higher in the ovaries of *P. esculentus* than in two other taxa. Primary oogonia were the main group of degenerating germ cells in all taxa, however in *P. lessonae* and *P. ridibundus* secondary oogonia and diplotene oocytes were also found. Significant strong positive correlation was found between gonadal stage and Gosner stage in *P. lessonae* and *P. ridibundus*. In *P. esculentus* the strong positive correlations were demonstrated between the Gosner stage and gonadal volume, and the number of degenerating germ cells, as well as between gonadal volume and number of degenerating germ cells. These observations suggest that increased rate of apoptotic changes of germ cells may be responsible for delayed maturation of ovaries in *P. esculentus*.

Key words: Hybridogenesis, *Pelophylax esculentus*, germ cells, apoptosis, ovaries
Tadpoles in personality research: testing current hypotheses of behavioural consistency using *Rana dalmatina* as a model

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Understanding the evolutionary and developmental background of behavioural consistency, i.e. consistent between-individual differences in behaviour, became a central topic in current evolutionary behavioural ecology. Although both animal personality (consistency in a single behaviour) and behavioural syndrome (consistency across two or more behaviours) have been proven in a wide range of taxa, amphibians remain notoriously underrepresented. However, anuran larvae (tadpoles) are common models in experimental evolutionary ecology and a few pioneering studies have recommended them for personality research, because they can be reared in high numbers under controlled settings, where it is easy to manipulate ecologically relevant environmental factors. Furthermore, tadpoles reach an important life-phase (metamorphosis) relatively fast, allowing the measurement of life history traits. Here, we report results from two common garden experiments on the agile frog *Rana dalmatina* tadpoles testing current hypotheses about behavioural consistency. We found evidence that agile frog tadpoles can develop animal personality and behavioural syndrome, but only when they are exposed to ecologically relevant stimuli, both from predators and conspecifics. Moreover, we found support for the pace-of-life syndrome hypothesis integrating behaviour with life-history variation. We discuss our findings in the light of present knowledge about the evolution and development of behavioural consistency.

**Key words:** animal personality, anuran, behavioural consistency, behavioural plasticity, behavioural syndrome, pace-of-life syndrome, tadpole
Detection and successful eradication of the invasive American bullfrog in the Netherlands

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Alien Invasive Species are considered to be a major threat to biodiversity. Effects on indigenous species may vary from hybridization, competition for resources, predation and the transmission of diseases. In the Netherlands most of the recorded invasive herpetofauna species are still in an early stage of the colonization process and therefore eradication efforts are expected to be effective. No American bullfrog (Lithobates catesbeianus) populations were known in the Netherlands since 1991. The species is however present in adjacent Belgium. Since 2009 an early warning system (EWS) for American bullfrog has been active in the southern part of the Netherlands. This EWS consists of monitoring specific ‘high risk’ sites, situated within 3 kilometers from Belgian bullfrog populations. The monitoring is conducted by volunteers. Additionally, environmental DNA is used to detect the early presence of cryptive bullfrogs. In 2010 a population of the species was discovered by a citizen, who was informed by our online documentation. This site was far away from the Belgian border. Eradication of this population started in 2011. Two different methods were used in the two reproduction ponds:

1. Enclosure of the pond, as well as complete draining and removal of vegetation, mud and bullfrogs.
2. Enclosure of the pond and the use of dip nets, seines and fykes to remove bullfrogs.

The results of these methods, both leading to the complete eradication, will be presented, as well as the key-factors for successful eradication. The key for success is not only a methodological issue, but is also based on effective communication strategies. Conducting risk assessments and developing EWS’s are crucial, since success of eradication is determined to a large extent by early detection. Specialized volunteers and the general public are extremely valuable in early warning systems.

Key words: Lithobates catesbeianus, Alien Invasive Species, early warning, elimination, eradication, citizen science
How to survive past climate changes – lessons from *Lyciasalamandra*

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The ten species of the viviparous salamander genus *Lyciasalamandra* are endemic to the Taurus Mountains and to some Aegean island. We reconstruct the range histories of all species using genetic data and species distribution modelling. A phylogenetic analysis (five mitochondrial genes, 4,500 bp) set a temporal framework of species evolution. Seven lineages evolved almost synchronously at Messinian times ca. 5.5 mya, with some of them showing a significant substructuring, presumably triggered by Quaternary climatic alterations. Within species, population genetic and phylogeographic patterns (based on two mtDNA markers, 1,000 bp) indicate repeated range expansions and contractions. Species distribution models using paleoclimatic data of the Eastern Mediterranean indicate that during periods of cold climate, suitable conditions for the species were met at low elevations, from where higher elevations could be re-colonised during warmer periods. Hence, *Lyciasalamandra* serves as a model for long-term survival of a highly specialised amphibian almost on the spot, with repeated small-scale range shifts allowing to survive periods of unfavourable climatic conditions.

**Key words:** Phylogeography, species distribution modelling, molecular clock, range shift, paleoclimate
Reconstructing the biogeographic history of geographically disjunct populations: the case of Salamandra s. bernardezi and S. s. giglioli

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The fire salamander, Salamandra salamandra, is a widespread species in Europe ranging from southern Iberian Peninsula to the Caucasus, and has an enormous variation in morphological and life history traits, with up to 16 described subspecies. Interestingly, two of these subspecies, S. s. bernardezi and S. s. giglioli, group in the same mitochondrial lineage while they show a clear disjunct distribution, being the former distributed along north-central Iberia and the latter occurring in southern Italy. To understand the biogeographic history of this group, and the environmental and ecological factors related to the separation of both subspecies, we here 1) identify probable suitable areas for both subspecies occurrence in current and past times, as well as 2) their stable climatic areas over time, and 3) assess the potential niche divergence between S. s. bernardezi and S. s. giglioli. Particularly, we aim to infer whether the distribution of the present populations of S. s. bernardezi and S. s. giglioli is due to persistence in separate Iberian and Italian refugia as response to Pleistocene climatic oscillations, or is the result from post-glacial recolonization followed by subsequent extinction of intervening populations. We used a well-resolved phylogenetic tree including representatives of all major clades in S. salamandra. Then we derived independent ecological niche models for each subspecies based on ca. 200 georeferenced samples analyzed with mtDNA (cytochrome b) and nuDNA (β-fibrinogen). We measured niche overlap based on bioclimatic variables. We projected the distribution of each lineage to the Last Inter Glacial (~130,000 yrs), to the Last Glacial Maximum (~21,000 yrs) and to the Mid Holocene (6,000 yrs).

Key words: biogeography, disjunct populations, ENMs, niche shift, Salamandra salamandra


_Cretaceous World_ – A 99 million-year-old lizard fauna in Burmese amber fills evolutionary gaps

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It was Plinius the Elder who reported first from a lizard preserved in amber. However, about 1900 years later the first “amber-lizard” was described as _Anolis electrum_ LAZELL, 1965. Since this times only a few other species were described from Caribbean (four species), Baltic (two species), Myanmar (one species) and Lebanon (one species) amber. The oldest fossils are single specimens found in Burmit (99 Myo) and Lebanon amber (120 Myo). Several new lizards were now found in Burmite amber representing a rich cretaceous squamate fauna. Compared with other fossils, e.g. indeterminate priscagamid remains from Mongolia (125.0–100.5 Ma), the oldest acrodont fossils from the Paleocene (65–56 Ma) and the oldest fossil chameleon from the Miocene of Africa (23–5.3 Ma), this assemblage fills an important gap in the evolutionary history of the squamates. Specimens were examined through the resin using Computed Tomography (CT) imaging. Morphological characters were added to a combined dataset of molecules and morphological data. Specimens were identified as members of the Lacertioidea, Scincidae, Gekkota and Acrodonta. Ongoing research will seek to describe in detail the morphology of the specimens to provide more data and to give a chance to include those fossils in evolutionary trees.

**Key words:** Burmit amber, Cretaceous, Acrodonta, Lacertioidea, Gekkota, Scincidae
Newts skip aquatic life and forego reproduction in response to alien fish introduction

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Many amphibians require both aquatic and terrestrial habitats during their life cycle. Among amphibians, newts are particularly aquatic as they usually court and lay eggs in water during several months. This makes them particularly vulnerable to fish introductions but mechanisms behind the exclusion patterns observed in the field are still not much known. Predation is one of the main reasons proposed to explain the absence or rarity of newts cohabiting with fish, but whether newts opt to skip breeding and leave water for land in fish environments has not been investigated yet. To test this hypothesis, we studied daily aquatic and terrestrial habitat use during the entire breeding season in a laboratory replicated design involving the palmate newt (\textit{Lissotriton helveticus}) and the goldfish (\textit{Carassius auratus}). In addition, we assessed sexual activity and reproductive success. There was a strong avoidance of the aquatic environment in the presence of fish, particularly when no aquatic shelter was available. Such an escape from the aquatic environment had a high negative impact on reproduction: in the presence of fish, newts displayed less courtship and laid very few eggs. The availability of aquatic shelters favoured coexistence between newt and fish but this did not prevent a large part of the newts to leave water and to skip reproduction. This experimental study shows how the presence of fish can cause newts to forego an essential part of their life – aquatic reproduction – and thus helps at the understanding of the exclusion patterns between fish and amphibians in the wild. More broadly, these data contribute to explaining aquatic versus terrestrial life in newts from fish and fishless environments.

Key words: amphibian decline, behavioural ecology, complex life cycles, conservation, fish introduction, habitat selection
Comparative Skeletal Osteology in Three Scincid Lizards (Genus: *Ablepharus*) from Turkey

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Despite the abundant and diversity of lizards in the family Scincidae, descriptions of their cranial and postcranial osteologies are almost nonexistent. Here, we provide detailed descriptions about adult skeletal morphologies of three scincid species: *Ablepharus kitaibelii*, A. *chernovi* and A. *budaki*. The skeletal elements of these lizards are described on cleared and double-stained specimens. The general pattern of the cranial and postcranial skeletons in three scincid species is characterized by the absence of the palatal teeth, presence of thirteen scleral ossicles in each eye, pattern of fore- hindlimb, and shape of the clavicle and interclavicle. But, there are some remarkable variations regarding the number, size and position of the bones. These include the size and shape of the nasal, the degree of fusion or separation of the postfrontal and postorbital and number of the presacral vertebrae and marginal teeth.

**Key words:*** Ablepharus, scincid, osteology
Do they compete or not? Using a combination of approaches to understand the competition between two similar lizard species

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Species of the same predator guild with similar ecological niches are likely to be in competition when living in syntopic populations. However, they can avoid competition by segregating in one or more dimensions of ecological niche when in syntopy. Furthermore, species possess a set of physiological traits that define their fundamental niche, which are expected to shape at least the temporal and spatial dimensions of their realized niche regardless the presence of other species. We compared two lizard species (Podarcis muralis and Iberolacerta horvathi) that exhibit an altitudinal segregation pattern with a high zone of overlap at middle altitudes, where syntopic populations occur. First we evaluated the potential for competition by comparing their morphology and fundamental niche characteristics. They highly resemble in morphology, mean preferred body temperature, water loss and metabolic rates, but show certain dissimilarities in seasonal acclimatisation of preferred body temperatures, microhabitat use and metabolic potential. Based on these results we used experimental thermal gradients to test for interference competition in thermoregulation. I. horvathi showed higher interference-susceptibility than P. muralis via agonistic social interactions. Also, common predators might influence the interaction between prey species via indirect competition. Results revealed asymmetry in anti-predator responses; while taking into account the costs of anti-predator behavior I. horvathi might be less competitive in areas of high disturbance. According to these results, behaviour and ecophysiology in combination seem to be so far the most important mechanisms contributing to the competitive outcomes between P. muralis and I. horvathi.

Key words: interspecific competition, ecophysiology, behaviour, Podarcis muralis, Iberolacerta horvathi
Divergence history of the Carpathian and smooth newts

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Information about the time of initial split, current and ancestral population sizes, and history of post-divergence gene flow between diverging species is essential for the understanding of the process of speciation. Gene genealogies are useful for testing various models of divergence and gene flow and estimate their parameters. We investigated historical demography and gene exchange between the Carpathian (Lissotriton montandoni) and smooth (L. vulgaris) newts. These sister species are morphologically, ecologically, behaviorally and genetically differentiated but hybridize in nature and introgression of some parts of the genome has been reported. Using Approximate Bayesian Computation (ABC) framework we evaluated several alternative hypotheses regarding the extent, direction and timing of genome-wide gene flow between these species. Inferences were based on 66 nuclear, non-coding markers (ca. 32 kb) collected from 58 populations. The model of recent (last glacial period) interspecific gene flow, allowing for demographic change during the Pleistocene was highly supported and favored over alternative models of i) isolation, ii) constant and iii) old gene flow. The estimated divergence time of ca. 4 mya suggests pre-Pleistocene species divergence, which is consistent with the fossil record. Our study indicates that despite the apparent long-term evolution in isolation the species not only retained the ability to hybridize but also that recent hybridization has led to genome-wide introgression, demonstrating that at the genomic level reproductive isolation is far from complete.

Key words: newt; Lissotriton; divergence history; gene flow
Analysis of phylogeny and introgression in small Eurasian vipers using RADseq data

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Small Eurasian vipers in the genus Vipera (subgenus Pelias) has about 20 described species and have been the object of recent molecular phylogenetic analyses. However, many species within the group are not supported by molecular results, demonstrate mito-nuclear discordance or frequently hybridize. We have applied Next Generation sequencing based restriction site-associated DNA sequencing (RADseq) to further analyse relationships among named taxa in these snakes. We sequenced representatives of each main group of small vipers and samples from all recently-identified major mitochondrial clades (V. anatolica, V. berus-complex, V. kaznakovi-complex (both northern and southern clades), V. renardi-complex, V. seoanei, V. ursinii-complex). The resulting tree that was generated using over nine hundred of independent loci with the coalescent-based method (SNAPP) supports our earlier mtDNA-based reconstruction with only minor exceptions: both clades of V. kaznakovi form monophyletic group and V. anatolica is a basal taxon to a clade containing V. ursinii and V. renardi. Additionally we checked by different methods (Bayesian clustering, multivariate analysis, Patterson D-statistics) the hypothesis of hybrid origin of two taxa from N-W Caucasus: V. orlovi and V. magnifica. All methods support a hybrid origin for these taxa and indicate they contain a majority of genetic material from V. kaznakovi with a small proportion of V. renardi genes. The hybrid status of these taxa could have impact on their conservation status. A similar proportion of genes from different species and low variation of admixture proportion values across specimens of V. orlovi from different localities could be result of stabilized hybrid population, which occupies particular habitat type. Hybridization could be an important source of new taxa in vipers and possibly contributes to local adaptation.

Key words: Vipera, RADseq, phylogeny, multilocus phylogeny, hybridization, evolution
SOSIguana – Saving the Lesser Antillean Iguana on St. Eustatius

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Globally there are only three small isles in the Caribbean region where the endemic Lesser Antillean Iguana (*Iguana delicatissima*) lives in a genetic pure form. St. Eustatius, one of the Dutch overseas territories is one of them. Therefore the species is severely threatened and on the IUCN Red List. One of the main threats is the hybridization with *Iguana iguana*, which was the reason of extirpation of *delicatissima* on several islands of the Lesser Antilles during recent decades. An Action plan for *delicatissima* is recently published (2014) and on Saint Eustatius implementation has been started immediately. The local nature conservation body STENAPA and the Dutch RAVON organization work together intensively. The focus is on raising public awareness (e.g. education on schools), research by population monitoring, capture-recapture and behavioural study, genetic study, a study to the fast growing invasive Corallita vine (*Antigonon leptopus*), which is covering over 20% of Statias natural vegetation in relation to the extent in which this affects *delicatissima*’s food supply. Preliminary results of these investigations and conservation measures will be presented.

**Key words:** *Iguana delicatissima*, Action plan, monitoring, capture-recapture, conservation measures, genetics (hybridization)
Preliminary monitoring of Bidder’s organ after castration of cane toad males *Rhinella marina*, as a tool of biological restriction of invasive species in Australia – pilot approach

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The males of cane toad were castrated in order to check whether in absence of androgens produced by their testes, the Bidder organ can develop into active female gonad being able to start oogenesis in thus sex reversed males. Such reversals after mating with normal males are expected to produce males only progeny, which could bias the sex distribution in wild living population and eventually exterminate females, thus finishing the natural reproduction.

The technic of surgical operation was worked out. After the first 2 months since operation the Bidder’s organs of castrated males, monitored with usage of USG image analysis has not changed their sizes. Also the typical males coloration and structure of skin warts remained the same together with releasing calls emitted after agitation.

**Key words:** Sex reversion, cane toad, Bidder organ
Poster presentations
Separate histories in both sides of the Mediterranean: phylogeny and niche evolution of ocellated lizards

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The ocellated lizards (genus *Timon*) show a disjunct distribution with two species each in south-western Europe, northern Africa, and the Middle East. They offer an interesting model to study the evolution of ecological niches through comparative phylolimatic analyses. Our study provides insights into the evolutionary history of *Timon* and helps to understand the role of environmental niche evolution during speciation processes. The Grinnellian niche of each species was characterized via species distribution modelling, using PC-derived environmental variables. A dated species tree was inferred from multi-locus molecular data. By combining the phylogeny and modelled niches, we reconstructed the ancestral environmental niche occupancy of each taxon using niche occupancy profiles. Niche divergence among species was quantified by computing multivariate niche overlaps via two-dimensional and n-dimensional approaches. Phylogenetic analyses support a vicariant divergence pattern with an early split of *Timon* into an eastern and a western group. A high complexity in the ecological niche evolution between the Mediterranean and non-Mediterranean climatic regions was detected. The generally low niche divergence among members of the eastern group and the remarkable divergence within the western group highlight an important role of temperature seasonality in the context of adaptation to Mediterranean and Atlantic versus continental climate. Our results also suggest niche conservatism in terms of microhabitats described by vegetation cover. The ocellated lizards provide an interesting example of a vertebrate radiation where niche shift (with or without vicariance) and niche conservatism alternate in different niche axes, shaping current biogeographic patterns.

Key words: Ancestral niche reconstruction, n-dimensional hypervolume, niche overlap, species distribution modelling, *Timon*, vicariance
Distribution modeling and Environmental Suitability of the Kelung Cat Snake
Boiga kraepelini Steineger, 1902

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Predictive potential distribution modeling is of increasing importance in modern herpetological studies and determination of environmental and conservation priorities. This study employs recently proposed modeling technique Maxent (www.cs.princeton.edu/~schapire/maxent) to analyze the geographic distribution pattern of Kelung Cat Snake Boiga kraepelini Steineger, 1902 in Taiwan Island and mountain part of Eastern and South-East Asia. We analyze the distribution patterns not only of the area in general, but for both parts of the range separately. Constructed model based on 63 localities within the territories of Vietnam, southern China, Laos and Taiwan identified dissemination of this snake species enough performance: for Vietnam AUC 0.94 with dispersion 0.031, for Taiwan 0.96 with dispersion 0.002 and AUC 0.99 with dispersion 0.001 for species range in total. The model results confirm the known distribution pattern of this species. The model however identified potentially suitable regions outside the known range of B. kraepelini in Yunnan, Sichuan, Guangdong, Fujian and Guizhou provinces, where it was mentioned by a number of experts, but without the exact localities. Comparison of relative importance of variables showed that temperature seasonality is the most important parameter in Taiwan and annual temperature scale is the most important parameter in continental part of distribution range. The identification of suitable areas for this species will help to assess conservation status of the species, and to set up management programs.

The study was supported by RFBR 14-04-92000_NSC) and by participation of Zoological Institute (№01201351184).

Key words: colubrid snakes, Maxent modeling, Boiga kraepelini
We studied cold tolerance in three species of frogs whose range extends to Yakutia, the coldest region of Eurasia. Since two of these species are considered endangered in the Red Book of Yakutia, the animals for the experimental study were collected in the adjacent territories (Middle Amur area and Novosibirsk).

*Rana amurensis* Boulenger, 1886 is distributed in Yakutia up to 71°N (Borkin et al., 1981). Cooling to -1.5°C was tolerated by all individuals (*n*=10); only one out of 10 tolerated cooling to -2.5°C; and at -3°C (*n*=10), all frogs died. However, cold tolerance does not limit the range of this species, since in Yakutia these frogs overwinter only in the rivers and non-freezing lakes.

*Rana dybowskii* Günther, 1876 penetrates to Southeastern Yakutia along the Aldan River valley and along the Okhotsk Sea coast. Cold tolerance of this species was similar to that of the populations of *R. dybowskii* from Northeastern China (Xiao et al., 2008) as well as to cold tolerance of *R. amurensis*. A limited range of this species in Yakutia is not due to its cold tolerance since it overwinters in water.

*Rana arvalis* Nilsson, 1842 penetrates only to Southwestern Yakutia along the Lena River valley. The specimens tolerated being frozen at -10°C over two days; at the same time, young frogs of this species from Denmark tolerate only one day exposure to temperature not lower than -4°C (Voiturron et al., 2009). Such a difference between Siberian and European populations appears to be a result not of different methods of study but rather of the geographic variation in cryoresistance, as described in a wood frog *Lithobates sylvaticus* LeConte, 1825 (Costanzo et al., 2013). The cold tolerance found in our study allows suggesting that *R. arvalis* in Yakutia overwinters on land.

**Key words:** *Rana amurensis, Rana dybowskii, Rana arvalis*, overwinter, cold tolerance, range
The role of minor landscape features for reptile conservation in agro-ecosystems

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Traditional agriculture had shaped European landscapes throughout the centuries leading, in many cases, to the creation of agro-ecosystems rich in biodiversity. However, as known, the intensification of agricultural practices, the large use of machineries and chemicals and the enlargement of the cultivated fields have led to the reduction and fragmentation of natural habitats, and to a gradual loss of biodiversity. Reptiles are threatened by agriculture intensification worldwide, but less is known about their distribution in agro-ecosystems, especially at field scale. We analysed reptile assemblages in an agricultural area dominated by intensively cultivated arable lands, in Central Italy: we compared abundance and diversity of reptiles among eight agricultural and semi-natural land uses and inside vegetated buffer strips intersperse among fields (i.e. strips of vegetation along ditch banks and field margins). Our results stress how intensive agriculture can hinder the presence of reptiles and highlight the key role of minor landscape features, like vegetated buffer strips, for the presence of vertebrates in intensive agro-ecosystems. Indeed, intensive crops and pastures hosted just one lizard species, semi-natural or less intensively managed agricultural land uses showed a better balanced faunal composition, while most of reptiles were recorded in the buffer strips. Interestingly, richness of individuals and species increased when strips were connected to semi-natural areas, independently from their width and vegetation structure. Such results suggest that the implementation of buffer strips should be encompassed among the measures for vertebrate conservation in agricultural landscapes.

Key words: Agriculture, Biodiversity, Reptiles
Visceral topography of the grass snake (*Natrix natrix*)

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Allometry – a study of how organism traits change with body size – is commonly studied in snakes. However, little is known about the allometry of internal organs in these animals. We describe topography of visceral organs and their allometry in the grass snake *Natrix natrix* from south-western Poland. Most of the studied organs show allometric growth. Gonads and kidneys grow with positive allometry in males and heart length in females. Negative allometry occurs in the length of tail in females, and length of trachea, heart and stomach in males. Notable differences between sexes include shift of anterior and midbody organs (except heart) towards the head and posterior displacement of kidneys in females and allometry in length of heart and tail. Males exhibit positive allometry in tail length and negative in heart length, while the converse is true for females. Population studied here differs from other closely located population in allometry of the tail length which may be an example of plasticity in some sexually dimorphic traits.

**Key words:** sexual dimorphism, internal organs, anatomy, allometry, snakes
Initial study on amphibian mortality patterns on railways

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Problem of amphibian railroad mortality is little known as opposed to road mortality. The current exploratory study reports one of the first empirical data in this area. The aim of the research was to quantify amphibian mortality along a railway line and to investigate the effect of the surrounding habitat and the seasonal variation of railway mortality of amphibians.

The study was conducted between April to July 2011 along 45 km of the railway line Kraków – Tarnów (Małopolska province, Poland). Dead individuals of *Bufo bufo*, *Rana temporaria* and *Pelophylax esculentus* complex were found within the area of the railway tracks. Furthermore, 77% of them were the common toads. The largest number of amphibian breeding sites was located in the most heterogeneous habitats (woodland and rural areas), which coincides with the sectors of highest amphibian mortality (42% of all accidents). As in the case of roads, spring migration seems to be the period of highest amphibian mortality (87% of all accidents) on railroad tracks. Railroad mortality seems to depend on physical features (such as body size, limb length) and may be associated with the agility of the species. Because of its physical features, the common toad was more likely to become stranded at the rail, indicating that this species is more vulnerable to railway mortality. The present findings show that railway mortality is a real threat for amphibians, an issue that requires deeper evaluation for conservation planning.

**Key words:** habitat effect, seasonality effect, common toad, agility
Taste organs in Gymnophiona – comparisons of distribution, size and morphology

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Previous research on caecilian taste organs demonstrated the presence of very few taste buds in the oral epithelium, while providing somewhat contradictory reports of their distribution within the oropharynx and among taxa. We investigated the distribution, size and morphology of taste organs of five caecilian species representing five families (Rhinatrematidae, Ichthyophiidae, Herpetidae, Indotyphlidae, Typhlonectidae) with light and scanning electron microscopy. Taste buds occur in the oropharynx of caecilians, in both larvae and adults. Our findings indicate that Gymnophiona differ from Batrachia (Anura + Urodela), which possess taste buds in larvae and taste discs in adults. The diameter of taste bud sensory zones ranges from 8.5 µm (in Idiocranium sp.) to approximately 14.0 µm (in Rhinatrema bivittatum), similar in size to taste buds observed in larval Batrachia.

Key words: caecilian, taste bud, sensory zone
Diversity of amphibians and reptiles in dynamic habitat – implications for conservation

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Human activity, next to habitat loss, can be a source of suitable environment for many species of amphibians and reptiles. Yet most anthropogenic habitats are dynamic and their properties for herpetofauna are appropriate only for some time. In our project, we aimed at investigating the diversity of human-originated habitats in limestone at different level of restoration. We have visited the Limestone Quarry “Górażdże” in Opolskie district 13 times during whole activity season in 2014. All data collected in the field were used for maps and spatial analysis in QGIS software. The inventory revealed the presence of seven amphibian species representing both anurans and caudates, and four species of reptiles, including rare smooth snake. The highest number of species, mostly reptiles, was recorded in medium stage of restoration, characterized by semi-open habitats. Areas in the initial stage of reclamation were inhabited only by eurytopic anurans. Habitats in late stage of restoration, mostly resembling natural and covered by dense forest, were inhabited mainly by amphibians, whereas reptiles exhibited decline. We conclude that for long term conservation of herpetofauna (with particular emphasis on reptiles), maintenance of semi-open habitats should be implemented in area management plans. We recommend preservation of semi-open habitats by constructing forest roads with edges without trees, preparing forest clearings, close to the forest roads. The maps and guidelines for reclamation were provided to the employees of the mine for further use.

Key words: diversity, habitats, reptiles, conservation, management, anthropogenic
Does facultative paedomorphism influence life history traits in the *Calotriton asper* populations?

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Morphology and life history traits were studied in a lacustrine population of Pyrenean newts (*Calotriton asper*) in the Ibón de Perramó (2,270 m.a.s.l.) in Central Pyrenees (NE of Spain). This population is composed of metamorphic and paedomorphic individuals (24.3% of gilled adults). We tested the effect of paedomorphosis on body size, shape, and age structure using skeletochronological methods. The body size showed significant sexual differences, being males larger than females. Paedomorphosis also affected the body size, suggesting a complex pattern of growth. In immatures, paedomorphs were larger than metamorphs, while the contrary situation was found in adults. The body shape was not influenced by the paedomorphosis, as well as the sexual dimorphism that was similar to those found in other populations. Skeletochronological results showed differences in the age distribution between sexes, with males (median age: 10.5 years) older than females (7 years), with a maximum longevity of 19 and 14 years, respectively. Considering each sex separately, the age structure was not significantly different between metamorphic and paedomorphic newts. In addition, we found metamorphic and paedomorphic immatures with a maximum age of 19 and 14 years, respectively. Remarkably, we found an exceptional larva of 18 years. The body size did not related to age with the only exception of paedomorphic females and immatures. We propose a hypothesis to explain this pattern of life history traits, based on the asymmetrical cost of metamorphosis, reproduction and growth in the population.

**Key words:** Facultative paedomorphism, Life history traits, *Calotriton asper*, Morphology, lacustrine population
Onset of amphibian monitoring programme in Latvia

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First amphibian monitoring efforts in Latvia had been carried out in 2008, using pitfall traps and vocalizing amphibian registration. That was a limited study carried out in 9 sites and had relatively poor success. Methodology was reworked in 2013, and pitfall method was dropped as too time and resources consuming. New improved monitoring methodology uses mainly vocalizing amphibian recording, with additional natterjack toad (\textit{Epidalea calamita}) and green frog (\textit{Pelophylax esculenta} complex) surveys. For anuran state monitoring programme in Latvia, a total of 70 squares with size of 5x5 km had been selected in randomly stratified way to represent all the main habitats and regions. In each square 12-15 water bodies potentially suitable for amphibians were selected for calling anuran record surveys. State financed pilot programme started in spring and early summer of 2015 in 20 squares, each was visited three times by professional herpetologists. Most often recorded species were \textit{Pelophylax lessonae}, \textit{P. esculenta}, and \textit{Pseudepidalea viridis}. First results indicated necessity to modify state approved monitoring methodology, and need for increase of visits to sites. During the monitoring pilot programme six young specialists were trained and first results about breeding habitat use by anurans in Latvia were obtained. The part of the preliminary research of vocalizing anuran in aquaculture was supported by project "Creation of a new scientific group for modernization of aquaculture technology" # 2013/0067/1DP/1.1.1.2.0/13/APIA/VIAA/060.

\textbf{Key words:} calling amphibian survey, monitoring, Latvia
First attempt on assessing the minimal population size of the Italian agile frog (*Rana latastei* Boulenger, 1879) in Slovenia

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The Italian agile frog (*Rana latastei* Boulenger, 1879) is an endemic species of the wider Padano Venetian plain area. It is one of the Europe’s most endangered amphibians, it is listed on Appendix II of the Bern Convention, on Annex II and IV of the EU Habitats Directive and it is globally vulnerable (VU) by the IUCN. Slovenian populations of the Italian agile frog are by far the best in Europe, due to both abundance and preserved habitats. In addition, high genetic diversity and resistance to disease makes them important for the long term survival of the species. Italian agile frog in Slovenia is distributed in western part of the country, in the regions of Kambreško and Goriška Brda (river Idrija with tributaries and right tributaries of river Soča) on the northwest, river Vipava Valley (river Vipava with tributaries) and river Branica Valley (river Branica with tributaries) on the west and Slovenian Istra (river Dragonja with tributaries and tributaries of river Mirna) on the southwest of Slovenia. This contribution is a first attempt to summarize data from different studies of the distribution and abundance of the Italian agile frog in Slovenia between 2003 and 2014. We obtained an insight into the population size and density in the study area by egg mass counting, the standard method used in all studies. The analysis resulted in an estimation of minimum population size of this species in Slovenia. Numbers are the highest in the population core area – western Vipava River valley, where approximately 12,000 egg spawns were counted on the area of 64 km² between 2003 and 2005. The rest of the Vipava River valley is intensively used for agriculture, where populations are unevenly distributed and rare, with most populations restricted to forest fragments around old oxbows. In 2011 more than 1,500 egg spawns were counted in such waters, while 170 egg spawns were counted on nearby isolated wetland Mlake in 2011, the easternmost population in Slovenia. The Mlake population is potentially connected with the Branica River valley, where between 860 and 1,400 spawns were counted in springs between 2003 and 2005.

Data on northwest (Brda, Idrija stream and Soča tributaries) and south (Istra, Dragonja River valley) range of Slovenian populations are scarce. Along the northwestern distribution just a few streams in Brda and a few localities in the river Idrija valley were surveyed in 2004, 2007 and 2009, where approximately 350 egg spawns were counted. Along the southern distribution, more than 400 eggs spawns were counted in a couple of localities in the Dragonja River Basin during a short herpetological field survey in 2012. As these two areas were never completely surveyed the numbers are certainly underestimated.

**Key words:** Italian agile frog, *Rana latastei*, minimal population size, Slovenia
Di- and triploid hybrids in *Pelophylax esculentus* population systems can reproduce by crossings with each other

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Interspecies animal hybrids survive and reproduce through clonal or hemiclonal reproduction modes. Such reproduction modes depend on endoreplication and elimination of parental genomes during gametes formation. Polyploidization enables recombination of clonal genomes during hybrid gametogenesis and allows hybrids to reproduce independently from parental species. European water frog complex (*Pelophylax esculentus* complex) represents suitable model to explore into polyploidization and hybrids maintenance in natural populations. The complex includes two parental species, *P. ridibundus* (RR genotype, 2n=26) and *P. lessonae* (LL genotype, 2n=26), which after crossing produce hybrid frog *P. esculentus* (RL genotype, 2n=26). Two forms of triploid hybrids (LLR or RRL genotype, 3n=39) also exist in natural populations. We asked how di- and triploid animals emerge and coexist with parental species. To answer this question we analyzed karyotypes of tadpoles appeared after artificial crossings of di- and triploid hybrids and *P. ridibundus*. We identified type of genome transmitted in growing oocytes of females participated in the crossings. We found that triploid hybrid females with RRL genotype produced oocytes with 13 bivalents and gave rise to diploid tadpoles. We suppose that oocytes of triploid hybrids with 13 bivalents formed haploid gametes with *P. ridibundus* genome. Some diploid hybrid frogs produced oocytes with 26 bivalents which presumably formed unreduced gametes and gave rise to triploid hybrids. Diploid hybrid males participated in appearance of *P. ridibundus* and hybrid tadpoles. We conclude that diploid hybrid males produced haploid sperm with *P. ridibundus* or *P. lessonae* genomes. To investigate genome elimination events we analyzed gonads dissected from hybrid and *P. ridibundus* tadpoles. In cytoplasm of germ cells only of hybrid tadpoles, we detected chromatin positive bodies (micronuclei) which contained eliminated genome according to earlier investigations. We conclude that triploid hybrids lead to appearance of diploid animals and can not reproduce independently from diploid hybrids.

**Key words:** Chromosome; European water frog; Hybridization; Karyotype; Tadpole; Oocyte
Connecting amphibian and reptile biodiversity: the case of LIFE “TIB - TRANS INSUBRIA BIONET” in Northwestern Italy

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Threats such as desertification, epidemics, pollution and climate change, generally ascribable to progressive habitat deterioration context, seem to be among the main causes of worldwide herpetofauna decline. Thereby, the set of actions proposed by LIFE “TIB – TRANS INSUBRIA BIONET” project, framework which includes the following measures that we present, is born from the need to tackle the loss of biodiversity and the degradation of habitats more and more fragmented, through enhancing the efficiency of the ecological corridor connecting the Alpine and the Continental bioregions in the Padanian-Insubric tract between the Ticino Valley and the Varese Prealps (Northwestern Italy). These actions consisted in a series of interventions aimed at increasing ecological connection of both reptiles and especially amphibians, class that several conservation biology studies pointed out to be among the most vulnerable to habitat deterioration. In this respect, between 2013 and 2014 two decayed wetlands have been restored and eight new ponds have been specifically created to facilitate amphibians’ reproduction. New dry stone walls have also been positioned in appropriately chosen areas and several now collapsed walls have been renovated to intensify the connectivity for reptiles in the entire territory. Finally, in order to limit the heavy damages produced by road traffic on amphibians populations during the breeding season, plastic barriers have been placed on upstream roadsides, interspersed by appropriate road underpasses in correspondence of highly frequented segments. Specific monitoring measures and phototrap-based surveys have then been carried out to confirm operation functionality. In this paper, we propose preliminary data on the use of such interventions in the short term by amphibians and reptiles populations, thus verifying their effectiveness in improving ecological connectivity in a heavily anthropized region.

Key words: ecological connection; amphibians and reptiles conservation; ponds and dry stone walls creation; road underpasses; Northwestern Italy
The Yellow-bellied toad (*Bombina variegata*) in South Tyrol (Italy): improved knowledge of its distribution and recommendations for an efficient conservation strategy of populations and habitats

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In South Tyrol the knowledge of the Yellow-bellied toad was incomplete, and lacking information on its status did not allow an effective conservation. Moreover, according to the last Italian report of habitats and species of European interest, its overall status was considered bad in the Alpine biogeographic region.

Our Project, promoted by Herpeton and funded by the Province of Bolzano and the Stiftung Südtiroler Sparkasse, started in 2013 and aimed to: update distributional data; evaluate conservation status of habitats; improve awareness and divulgation; provide technical management suggestions for conservation of populations and habitats, both by restoring existing aquatic and terrestrial sites and creating new ones. The field research methods were: Systematic Sampling Survey Area constrained (Plot and line transect), Visual Encounter Survey, active research under refuges, call survey, tadpoles sampling and clutch survey. Our results provided a picture of the distribution and the conservation status of *B. variegata* in South Tyrol, and highlighted areas not yet covered, to be investigated in the future.

The Yellow-bellied toad localities (50) were located within 15/109 (13.8%) UTM squares 10x10 km, mainly in the valley floor of Adige. It also inhabits Isarco, Venosta, Pusteria and Aurina valleys and some mountain areas in the southern part. The altitudinal range was 211-1535 m a.s.l. (39.2% under 500 m a.s.l., 43.1% above 1000 m a.s.l., 17.6% intermediate elevations). The species is mainly linked to precarious habitats in agricultural lands, peat bogs or pasture areas. It inhabits both lotic permanent and lentic permanent or temporary water, like streams, small canals, ponds, drinking troughs, flooded meadows, small dips, wheel groove. We identified several threats and found that protected areas do not always assure the persistence of populations. Therefore, we provided specific technical recommendations to improve both terrestrial and aquatic habitats, and to promote an ecological network by means of stepping-stones to connect the areas.

**Key words:** Yellow-bellied toad, distribution, threats, active conservation, South Tyrol
Effect of environmental temperature on body condition and jumping performance of two geographically distant *Rana temporaria* populations

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Environmental temperatures affect all physiological processes and performance of ectotherms. Performance directly influences fitness by determining the probability of survival until maturity. Therefore, physiological adjustments to local thermal environment are extremely important for achieving optimal performance levels. *Rana temporaria* is a widely distributed European frog, present in a variety of habitats. This makes it an exceptional model for studying population-specific physiological adjustments to the local environmental conditions. We hypothesized that *R. temporaria* populations from colder (Central Europe) and warmer (Southeast Europe) environments show population-specific variation in developmental time, morphometric traits and jumping performance, as a consequence of differences in local thermal conditions. We raised tadpoles from two populations under the same set of developmental conditions – in two constant temperature treatments, and in a semi-natural garden setup. After metamorphosis, we compared the influence of environmental temperatures on duration of developmental period, morphometric traits and jumping performance among froglets from different treatments, within and across populations. We detected significant effect of developmental temperatures on all measured traits. Moreover, we found significant differences in those traits between different populations, indicating that reaction mechanisms to environmental temperatures are population-specific.

**Key words:** amphibian, frog, environmental temperature, performance, climate change
Intraspecific differentiation in spatial distribution along the latitudinal gradient in *Vipera ammodytes*

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This study explored the intraspecific spatial relationship among the three clades of *Vipera ammodytes* in Western Bulgaria. They contact in few points along the Struma and Iskar river basins. Five polygons were chosen along the south-north gradient of the species distribution. They were explored monthly between 2013 and 2015, during the active period of the animals. Three were situated outside the contact zones (in Kresna gorge, Balsha and Lakatnik) and the other two were at locations of contact between the southern/northern green-tailed clades with the red-tailed clade (related to the nominate subspecies) (Bosnek, Karlukovo). Data from other parts of Western Bulgaria, collected haphazardly, have also been included in the analyses. Snake localities dataset was processed with multi values extraction function from geographic and climatic layers in ArcGIS. Attributive data were processed with factorial analysis. Preliminary results revealed that in both places previously considered as contact zones (based on older distribution data), the nominate subspecies has been completely displaced by the green-tailed clades. Diurnal temperature amplitude and annual precipitation as well as their seasonal expressions significantly influence and determine the spatial distribution of the designated groups. All compared groups were separated well by climatic instead of geographic variables. For example, all three clades were confined to slopes with predominantly southern exposure. Complex climate changes seem to influence and benefit range expansion of southern and northern green-tailed clades instead of the red-tailed clade in the zones of contact.

**Key words:** contact zones, climate changes, intraspecific competition
Does ecophysiology determine reptile responses to fire regimes? Evidence from Iberian lizards

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Reptiles are known to be sensitive to habitat disturbance, including fires. However, evidence from systematic monitoring suggests that reptile species show opposing responses to fire. In particular, in a contact zone between Mediterranean and Atlantic bioregions in N Portugal, field transects indicate that Mediterranean species (i.e. *Timon lepidus*, *Psammodromuns algirus* and *Podarcis guadarramae*) increase in abundance in burnt areas whereas Atlantic species (i.e. *Lacerta schreiberi* and *Podarcis bocagei*) decrease. Here we test if such differences across species are of functional nature, namely, if thermal and hydric ecophysiology may determine their response to fire. The habitat structure greatly differs between unburnt and repeatedly burnt sites. For this reason, we hypothesise that those species favoured by frequent fire regimes should be more thermophile and suffer lower water loss rates than those negatively affected. We collected 8-10 adult males of each species from N Portugal in spring 2015 and measured their body mass (BM) and length (SVL). We then submitted them to standard experiments for assessing preferred body temperatures (Tₚ) and evaporative water loss rates (EWL) and examined the variation of both parameters between species and along time by means of repeated-measures AN(C)OVAs. Results only partially supported our initial expectations. The medium sized *P. algirus* clearly attained higher Tp and lower EWL as expected. The two small species (*P. bocagei* and *P. guadarramae*) displayed low Tp and high EWL while the two large species (*T. lepidus* and *L. schreiberi*) displayed intermediate values. However, the predictions of finding differences within each pair were not confirmed. Moreover, although EWL decreased with lizard size within species, results between species remained similar after introducing lizard size as covariate in the analyses. We conclude that ecophysiology may help to understand reptile responses to fire but other factors like habitat selection are to be considered as well.

Key words: lizards, fires, functional response, ecophysiology, habitats, water-loss rates, preferred temperature
Detecting adaptation in immune genes of closely related, hybridizing amphibians

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Amphibians face severe declines due to globally spreading viral and fungal diseases. Pathogens are therefore expected to exert strong selective pressures on immune genes in this group of vertebrates, leaving detectable footprints in genomic data. There is an abundance of studies investigating amphibian MHC genes, which are responsible for pathogen recognition and trigger immune response in vertebrates, or antimicrobial peptides, a part of skin defense against different pathogens. However, studies looking on a wider spectrum of immune genes are scarce and a complete molecular analysis of immunological repertoire in amphibians has not been conducted so far. Here we used a targeted re-sequencing approach to scan more than 600 immunological genes in two closely related species of newts for signals of adaptive evolution with an emphasis on balancing selection. The two species: Lissotriton vulgaris and montandoni constitute a complex of recently diverged, morphologically distinguishable taxa, characterized by a complex history of hybridization. To overcome the confounding effects of demography and population structure in these species, a null demographic model was derived from neutral sites and appropriate sampling scheme was applied. We used several population genetic approaches to detect plausible targets of balancing and positive selection, and also described and contrasted the selective regimes operating in the immune genes of the two taxa. Identification of loci under selection is a first step to study host-pathogen interactions on a spatial scale, and to infer the impact of gene flow on spreading beneficial variation between species and populations.

Key words: genomic scans, adaptation, balancing selection, immune genes, Lissotriton
Ziraba: a multidisciplinary project towards the conservation of herpetofauna

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Historically, humans have repudiated reptiles and amphibians, which are the most endangered vertebrate groups. Some species, such as snakes, suffer direct persecution, and monitoring and conservation initiatives are seldom launched from the institutions. The project Ziraba was launched to stimulate a shift in the perception of the society towards this faunal group. The hostility against herps is largely motivated by unfounded myths and legends. Informing about the herpetofauna rigorously and bringing the animals closely to the people are powerful tools to refute these myths. The project developed in the municipality of Hernani (ca. 19600 people) is a pilot intended to be expanded to other areas in next years. Different initiatives have been driven during the last three years (2013-2015) in favour of herpetofauna. On the one hand, an exhaustive inventory (up to 600 records) of the herpetofauna from Hernani was completed. For this initiative apart from records from herpetologists, contributions from citizens (35 people providing 96 new records) were received. On the other hand, a pedagogical work has been performed with 2-10 years-old children. Children worked for three months in the school on a teaching unit focused on amphibians. Children took part on samplings, interviewed older people to obtain historical data and prepared theatres, workshops and exhibitions. Finally, apart from the inventory and pedagogical work, a network of 15 ponds has been constructed to enhance the connectivity between the two Special Areas of Conservation, the Urumea River and Aiako Harria.

Key words: Ziraba project, Amphibians, Reptiles, Pond network, Hernani, Special Areas of Conservation, Urumea, Aiako Harria
Numerous studies indicate that different aspects of animal locomotor performance are ecologically relevant in different structural habitats, as locomotion is frequently required to capture prey, escape from predators, thermoregulate, find mates and defend territories. For this reason, lizards have been extensively used as model organisms in ecomorphological studies focusing on maximal locomotor performance as the link between spatial ecology and phenotypic characteristics. Typically, maximal value of performance is quantified across distance intervals of different length, depending on the study organisms. Here, we analyze how this choice may influence statistical inference and biological interpretations derived.

For this purpose, we measured sprint speed in 153 individuals of *Podarcis bocagei* by chasing lizards along a 1m-long horizontal surface with a cork substrate. All trials were filmed at 50 frames per second, the position of the lizard across each trial was digitized frame-by-frame and the maximum performance capacity was obtained using two alternative methods: 1) maximum instantaneous speed between frames and 2) maximum speed across any 10-cm interval. Our results show that the data obtained using 10-cm intervals were categorized, not normally distributed, exhibited lower individual repeatabilities and had four-fold increase in total variance compared to the data obtained by quantifying instantaneous speeds between frames. These results have important methodological implications, as they suggest that the choice of the interval across which speed is recorded substantially influences the quality and bias of maximal performance descriptors. This may in turn have a profound influence on ecomorphological inference, as it affects our capacity of detecting variation in performance within and across groups, and associations between locomotor performance and other traits.

**Key words:** Locomotion, Performance, Methodology, Sprint capacity, Lacertidae
Erythrocyte size and asymptotic body size in two populations of the sand lizard (*Lacerta agilis*) – does predation matter?

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According to the theory of optimal body size, mortality risk (e.g. caused by predation) promotes earlier maturation at smaller body size. What is more, high predation populations should evolve physiological capacity to escape predators. According to the theory of optimal cell size, one component of this capacity might be smaller cells that form a body. Small cells provide better transport capacity of tissue because of their relatively large cell membrane area. We studied sand lizards *Lacerta agilis* in two populations in the southern Poland. One population inhabited an open meadow (high predation) and another population was located in a meadow surrounded by dense forest (low predation). We hypothesised that the size of erythrocytes and the asymptotic body size would be smaller in the high-predation population. We found that lizards from the open meadow more frequently suffered tail loss compared to lizards from the forest meadow (p=0.017), which supports our hypothesis about the link between predation and habitat type. Nevertheless, lizards in the two populations had similar growth trajectories and the size of erythrocytes, against expectations based on the theory of optimal body size and cell size. Also, we found no relationship between body size and erythrocyte size, which suggests that developmental increase in body size is caused in this species by an increase in cell number rather than cell size. We conclude that despite apparent differences in predation pressure, life history and cellular characteristics of the studies lizards remained invariant. It is likely that the pressure of predators in the studied populations is weak relative to other components of mortality, which would explain why links between predation and life history and cellular traits were undetectable.

Key words: lizards, predation, life history, cell size, body size, growth
Egg predation of European Pond Turtle (*Emys orbicularis*) in Ipoly-valley, Hungary

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Strong population of the European pond turtle (*Emys orbicularis*) occurs in the river Ipoly, a natural borderline between Hungary and Slovakia. Only a limited 8 ha sand-hill area provides suitable sites for egg-laying for turtles, where strong nest predation was observed in previous years. In 2013 we started a project, funded by Swiss Contribution, focusing on the problem: to record predated nests and also try to develop methodology to safeguard untouched nests. In 2013 and 2014 we recorded annually over 200 nests, laid during May and June, 95% of them instantly predated with an average of 10 eggs per nests, where egg-shells remained in the vicinity of the nest. By using camera traps and analysing foot-prints we concluded that main predators are Red fox (*Vulpes vulpes*) and badger (*Meles meles*), whose numbers might have grown significantly during last decade due to immunisation programme against rabies. We also recorded the presence of other potential predators like wild boar (*Sus scrofa*) and European pine marten (*Martes martes*). In order to save untouched nests we applied steel netting over 25-30 nests in both years. In 2013 we used 30x30 cm pieces of steel net with 5x5 cm spacing. After a while some of them became predated, showing quick adaptation by predators, mainly foxes. In 2014 we used larger, 50x50 cm pieces with denser 3x3 cm and 2x2 cm spacing. It turned out to be much more successful and none of the saved nest was predated. This way we managed to head-start juvenile turtles, helping over 200 individuals to reach the water in 2014. Over these two years we observed that majority of the juvenile turtles emerged from their nests in March, hatching already in September, but overwintering in the nest chamber.

**Key words:** European pond turtle, egg predation, camera trap
Next Generation Sequencing of reptile parasites: methodological issues

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Despite the clear importance of parasites, both as components of biodiversity and due to their impact on host species, a lack of basic knowledge of most parasite groups hinders risk assessments and conservation planning. This is particularly true for non-model host groups, such as reptiles. Recently the use of molecular tools for parasite screening, identification and phylogeny reconstruction has become more widespread. However, for many parasites few molecular markers are available, and most studies are based on data from single genes. With the advent of next generation sequencing techniques, it is possible to develop vast numbers of markers to gain better assessments of parasite diversity. However, unique problems arise, in particular the difficulties in removing host DNA, the issues of obtaining a large enough quantity of DNA and the lack of comparative data from public databases. Here we discuss how we overcame some of these problems in two different parasite groups, Apicomplexan blood parasites (Hepatozoon sp.) in Iberian Podarcis lizards, and various nematodes from Gallotia sp. from the Canary Islands.

Key words: Hepatozoon, Apicomplexa, Nematodes, Podarcis, Gallotia
Development of the dentition in the grass snake *Natrix natrix* L. (Lepidosauria, Serpentes) embryos

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Dental patterns in vertebrates range from absence of teeth to multiple sets of teeth replacing throughout life. The first morphological sign of the tooth formation is a thickening of oral epithelium at the site of the future tooth row, so called the primary dental lamina. Then, tooth develops from bud, through cap to bell stages. In comparison with mammals, much less is known about dental development in other vertebrates, especially in reptiles. The aim of this study was to describe development of the dentition in the common species of snake in Poland – the grass snake (*Natrix natrix* L.) embryos. The structure of the teeth in the consecutive developmental stages has been studied using standard histological techniques. The age of embryos was calculated using the developmental table for this snake species. At the time of egg-laying on either side of the maxilla were present teeth buds as dental lamina. These continuous invaginations of oral epithelium into the dental mesenchyme gave rise to all the teeth. Invaginations, initially little, elongated during following developmental stages and at the stage VI were present both in the maxilla and mandibula and the first teeth at cap stage were observed. The epithelial cell population proliferated and differentiated into the enamel organ which produced ameloblasts. Instead, the mesenchymal cells differentiated into dental papilla and gave rise to odontoblasts, which produced dentin. A dentin started to form in the late stage VII of development. At the time of hatching all teeth were well developed. The best developed teeth (cuspidal in shape) were observed at the anterior part of the maxilla. Enamel was not observed in any developmental stage. The new teeth were still forming. Both the structure of teeth and mode of their formation were similar to those in mammals and other reptiles.

**Key words:** reptiles, grass snake, dentition, development
Effects of drought and potassium loading on osmotic balance in the Souss valley tortoise *Testudo graeca soussensis* in an arid area of West central Morocco

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The Souss Valley tortoises, *Testudo graeca soussensis*, occupies various habitats in west central Morocco, including arid environments where they are frequently confronted to unpredictable chronic droughts. These can affect their water and osmotic balances due to the lack of water and high salt concentrations in food plants, especially potassium. Seasonal patterns of osmoregulation were investigated during different periods between April 2011 and March 2012 in an arid area of West central Morocco. Plasma osmolality in free-ranging tortoises remained relatively constant across seasons, but not urine osmolality which showed significant seasonal changes due to variation in water availability related to chronic drought and occurrence of rainfall events. Dehydrated adult tortoises (9 males and 8 females) which experienced 6-8 weeks of drought were captured, weighed, bled and their voided urinary fluid occasionally collected. They were then kept in captivity and rehydrated while provided with a mixture of fresh vegetables and lucerne and water *ad libitum* for two weeks. They were then reweighed, rebled, and their voided urinary fluids taken 24h before and after an acute KCl overloading. The plasma and urinary fluid osmolalities, electrolytes’ (sodium, potassium and chlorine) and total nitrogen concentrations were measured. The urinary fluid/plasma osmolality ratio, approaching isotonicity, in both dehydrated and potassium overloaded tortoises was much higher than in rehydrated ones (respectively 0.80 and 0.86 vs. 0.24). These were indicative of an advanced dehydration condition in free-living tortoises the urinary electrolytes’ concentrations of which were relatively higher and comparable to those in potassium overloaded tortoises. These showed a relatively higher urine total nitrogen concentration. Implications for conservation are discussed with respect of the PEP (Potential Excretion of Potassium) hypothesis (Oftedal, 2002) according to which plants high in water and/or proteins, but low in potassium should be selected in favorable periods.

**Key words:** *Testudo graeca*, dehydration, electrolytes, potassium, osmoregulation
Positive covariation between blood parasite infection and risk-taking in male Carpetane rock-lizards (*Iberolacerta cyreni*)

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Determination of the evolutionary and developmental factors underlying consistent behavioural differences between individuals is the main goal in the field of animal personality studies. Since estimating fitness is often challenging, linking animal personality to individual quality is not straightforward in most cases. Here, we studied behavioural consistency and the link between behaviour and potentially fitness-related traits in wild caught adult *Iberolacerta cyreni* males during the mating season. Activity and risk-taking behavioural types (individual mean behaviour) were evaluated based on five and six repeated assays, respectively. We found that both activity and risk-taking are repeatable within-individuals, suggesting the presence of animal personalities during the mating season. We also found a strong positive correlation between blood parasite (Haemogregarinidae) infection intensity and risk-taking. Considering that haemogregarines have relatively mild negative effects on their final hosts, this result is in line with the asset protection hypothesis, where individuals with decreased future reproductive value are expected to invest more into current reproduction, adopting a higher risk-taking behavioural strategy.

**Key words:** animal personality, behavioural type, within-individual behavioural variation, individual quality
Contact zone of two genetically distant groups of adder (Vipera berus) in southern Germany

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In SE Bavaria, the ‘Alpine clade’ of Vipera berus meets V. b. berus. Based on mitochondrial DNA, both have a high genetic distance which is explained by different geographic origins. The ‘Alpine clade’ probably originated south of the Alps where it also survived during glacial periods. We studied the German part of the putative contact zone, notably 3 populations, by a comparative microsatellite analysis. Samples from western Bavaria and from Austria were also included. 7 microsatellite loci were polymorphic and showed population-specific patterns. However they did not follow the dichotomic mitochondrial pattern. These results are discussed in the light of past and present gene flows between populations. Fragmentation and inbreeding are complicating factors but must be taken into account. They disturb the evolutionary interpretation, but as adders are becoming rare, these factors are getting important for conservation of the species and its intraspecific diversity.

Key words: Vipera berus, Alpine clade, genetic diversity, microsatellites, gene flow
Are alien tree plantations ecological traps for amphibians? Studying the impact of eucalyptus plantations on Iberian amphibian larvae

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On the Iberian Peninsula one of the most abundant anthropic habitats are eucalyptus tree plantations. Although many studies agree that tree plantations are detrimental for biodiversity, very few studies focus on their effects on amphibians. In addition, most of studies examine species richness or species abundance, but not one examines the effects of tree plantations on the ecology of the species that inhabit them. This is an important issue because tree plantations may be acting as ecological traps that affect fitness and finally lead these species to extinction. The aim of this study is to assess the role that the replacement of native forests by eucalyptus plantations has on some important aspects of the ecology of anurans. From previous studies, we know that some amphibian species can be found in eucalyptus plantations, but, although they are able to survive, we do not know the cost these paid for it. Thus, we performed the study on two species that can be found in eucalyptus plantations (Rana temporaria and Pelophylax perezi) and we measured the following variables on tadpoles maintained under controlled conditions in mesocosms with eucalyptus or oak (control) leaf litter: size and tail shape after 21 days of experimental exposure; innate immune response (using phytohemagglutinin test); and, finally, size, tibia-fibula lengths and jumping ability at metamorphosis. In both species, eucalyptus exposed individuals showed lower immune response, and worse body condition and reduced jumping ability after metamorphosis. However, we found no differences in larval growth or metamorphosis size. Results suggest that the eucalyptus negatively affect some aspects that may reduce survivorship and fitness in these species, which supports the ‘ecological trap’ hypothesis.

Key words: ecological trap, eucalyptus plantations, anthropic habitats, immune response, Rana temporaria, Pelophylax perezi
The older, the better? Effect of pine plantations’ age on the immune response and secondary sexual characters in the palmate newt (*Lissotriton helveticus*)

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Natural habitats have been conversed to anthropized environment on an unprecedented scale. The new simplified habitats have different selective pressures comparing with natural ones, so they are exploited by species adapted to them, which can tolerate such changes. The palmate newt (*Lissotriton helveticus*) has a wide distribution, often appearing in eucalyptus and pine plantations. In previous studies we found that newts living in eucalyptus plantations have always worse condition (lower immune response, worse body condition and less developed sexual characters) than those from natural forest, but the results were more variable on pine plantations: some of these were similar to eucalyptus plantations, while others were more similar to natural forests. Some studies have found that biodiversity in old pine forests is more similar to that found in native forests, so we wonder whether the age of pine plantations could also explain the previously observed differences. Thus, we captured 20 male palmate newts in each one of 12 pine plantations in the French region of Aquitaine (a total of 240 individuals from 4 plots of young pine forest, 4 plots of middle age and 4 plots of old age). We measured immune response (by the phytohemaglutinin assay), body condition and sexual characters (tail and hind feet webs area, and caudal filament length). The results showed that the age of the plantation influences measured characteristics and newts in better conditions were those from young and old pine plantations. We discuss the results in relation with forestry management. Alignment of forestry and nature conservation policies remains a challenge. Our results will provide useful information for integrating forestry and amphibian conservation in the context of agri-environment schemes.

**Key words:** Anthropic habitats, alien tree plantations, pine plantations, immune response, forestry management, amphibian conservation
Impacts of climate change on the global invasion potential of the African-clawed Frog *Xenopus laevis*

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The African-clawed Frog *Xenopus laevis*, native to the Sub-Saharan African region was distributed to laboratories all across the world as a model organism and medical purposes. The species possesses a strong invasion potential and populations have become established in numerous countries across multiple continents. Invasive amphibian species can have devastating impacts on native ecological communities by altering or eliminating delicate ecological relationships. Recent research revealed the invasive *X. laevis* populations to have negative impacts on local amphibian and fish populations. In order to determine the present and future global invasion potential of *X. laevis* we first computed correlative species distribution models based on a comprehensive set of occurrence records from South Africa, North America, South America and Europe and nine environmental predictors with a spatial resolution of 2.5° minutes using both an ensemble approach integrating eight algorithms and the Maxent approach. Future occurrence probabilities for *X. laevis* were subsequently computed using bioclimatic variables for 2070 following four different scenarios which were recently proposed by the Intergovernmental Panel for Climate Change (IPCC). These predictions suggest decreasing occurrence probabilities in the native range in South Africa and in invasive populations from North and South America, but a trends of increasing occurrence probability in Europe.

**Key words:** climate change, extrapolation, global warming, invasive species management, species range, species distribution modelling
Comparative study of skeletal development of *Microhyla ornata* (Anura: Microhylidae) and *Duttaphrynus melanostictus* (Anura: Bufonidae) during metamorphosis

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With a few exceptions, anurans have biphasic life cycles in which the aquatic larval forms metamorphose into terrestrial juveniles. Formation of the adult skeletal system is a complex process that is initiated during metamorphosis. Adults of different anurans show subtle differences in the skeletal elements though basic skeletal pattern remains the same. In the present study, we describe comparative aspects of skeletal development in the members of two families, *viz.* Microhylidae and Bufonidae during metamorphosis. *Microhyla ornata* and *Duttaphrynus melanostictus* are common and widely distributed in India. Tadpoles of both the species were fixed in 10% formalin, at Gosner stages 28, 32, 35, 38, 40, 42, 43, 44, 45 and 46 and subsequently stored in 70% ethanol. They were stained with alcian blue-alizarin red double staining method. The stained tadpoles were photographed and comparative skeletal development was studied to understand the similarities and differences in the skeletal elements and ossification. In *M. ornata*, chondrocranium appears at stage 35 while, in *D. melanostictus*, it appears at stage 28. Further, in *M. ornata*, hyo-branchial apparatus is large and modified into a branchial baskets whereas it is smaller in *D. melanostictus*. Pectoral girdle of *D. melanostictus* is typical of anurans, while omosternum and clavicles are absent in *M. ornata*. All vertebrae except atlas have conspicuous transverse processes in *D. melanostictus* while in *M. ornata*, the transverse processes of 5th, 6th, 7th and 8th vertebrae are very small. Iliac rods of the pelvic girdle extend much beyond the transverse processes of the 9th vertebra in *M. ornata* while in *D. melanostictus*, they are shorter. The overall ossification is completed by stage 43 in *M. ornata*, while it does not complete even at stage 46 (completion of metamorphosis) in *D. melanostictus*. These similarities and differences are discussed in the context of their ecology.

**Key words:** Skeletal Development, Microhylidae, Bufonidae
Thermal benefits of activity on road surface – case of Spadefoot toad
*Pelobates fuscus*

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In 2013 and 2014, amphibians along three road transects in NW Poland (23, 26 and 28 km, respectively) were counted between July and October. The transects consisted of both paved and unpaved public roads as well as short sections of forest tracks. We measured the temperatures of amphibian skin and road surface in the spot where the animal was found. Additionally we measured the temperature of the road verge (0.5 m from the road surface), ground 6 m from the road verge (mostly it was the ground temperature in the crop) and air temperature. We also noted the position of the amphibian on the road surface (sections: road verge, road edge, lane edge, lane centre, road centre). Among 936 amphibians found (685 alive, 276 dead), the Spadefoot toad *Pelobates fuscus* was the most abundant (264 alive, 130 dead individuals). Spadefoot mortality on unpaved roads was much lower than on paved roads (5 dead : 108 alive vs. 124 dead : 139 alive, respectively). Spadefoot toads, both dead and alive, were most often found on lane edges, i.e. areas with the highest probability of hit, and the differences in abundance between road sections were significant in all cases (chi-square tests – dead/unpaved: dead individuals present on lane edges only; alive/unpaved: chi-square=41.159, p<0.001; dead/paved: chi-square=270.387, p<0.001; alive/paved: chi-square=33.875, p<0.001). Toads found on roads maintained higher skin temperature than ambient ground temperature, and the difference was more expressed on paved roads (Wilcoxon test – unpaved roads: W=(-7.694), p<0.001; paved roads: W=(-9.425), p<0.001). Thermal benefits (t_{skin} – t_{ground}) were not significantly different among road surface sections (Kruskal-Wallis ANOVA – unpaved: K-W=1.449, p=0.836; paved: K-W=3.631, p=0.458). Activity on the road surface provides clear thermal benefits for *P. fuscus*, but their variation between road sections is not enough to explain the abundance pattern of the toad. We hypothesize that some other factors, e.g. invertebrate food availability, could attract those amphibians to the most dangerous part of the road.

**Key words:** road ecology, thermal ecology, road mortality, *Pelobates fuscus*
Do Anguis fragilis L. and Anguis colchica (Nordmann, 1840) hybridize in the south central part of Poland? Morphological signs of introgression between two species of slow worm, and range of Anguis colchica in Poland – preliminary results

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Recent nuclear and mitochondrial DNA analyses revealed 5 clades within genus Anguis which were elevated to the species level. However, hybrid specimens between some of these taxa were detected. Two slow worm species occur in Poland: A. fragilis and A. colchica incerta. Still little is known with regard to the exact distribution of contact zone of these two forms. However, the occurrence of A. fragilis is associated with the western part of the country whereas the occurrence of A. colchica is associated with the eastern part of the country. It appears that the western border of A. colchica in Poland is Wisła River, but some data show that is possible that the species is crossing that river, and its range is wider. The specimens from a number of populations in Poland were measured. Morphometric (obtained by “traditional” and truss network measurements), meristic and qualitative characteristics were used in the research. Study revealed intermediate morphotype of some populations. This may reflect occurrence of a hybridization zone and the existence of introgression between A. fragilis and A. colchica. In this study we discuss these two explanations of observed morphological differentiation and propose a distribution of contact zone between two species of slow worm in Poland.

Key words: Anguis fragilis, Anguis colchica, slow worm, morphology, hybridization, introgression, biogeography
Non-native amphibians pet trade via Internet in Poland

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Overharvesting and trade of amphibian populations is one of the reasons of their global decline. Trade via the Internet not only allows purchase of increasing number of rare and endangered amphibian species from around the world but it also influences the spread of invasive species. The aim of our research was to investigate the amphibian pet trade in Poland conducted in stores or on online portals. Between November 2013 and October 2014 regularly, once a month, we checked offers of sales on the website’s of 18 the biggest pet shops in the country which specialize in exotic animals, on a nationwide auction portal and on 3 exotic pet fans portals. During the study we reported 486 offers of 112 amphibian species on online stores and portals. Most of the offers were related to 4 families of amphibians: poison dart frogs – Dendrobatidae, tree frogs – Hylidae, true toads – Bufonidae and salamanders – Salamandridae. Our data show increased interest in amphibians as pets in Poland. Important is that trade of amphibians can be one of causes of the spread of diseases such as chytridiomicosis. More than half of offered species including 8 from the top ten of available species in Poland may be vector of Batrachochytrium dendrobatidis.

Key words: wildlife trade, exotic amphibians, invasive species, Bd, CITES, Poland
Digit ratio in common toad *Bufo bufo*

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Digit ratio (2D : 4D) denotes the relative length of the second and fourth digit. It is considered to be a suitable biomarker of the *in utero* balance of fetal sex hormones, which affect the early development of an individual’s morphological as well as behavioral characteristics. In recent decades, digit ratio attracted a great deal of attention in biology and psychology. However, for unmasking the biological basis of the phenomenon, extensive studies in non-human animals are necessary. It is generally assumed, that in mammals and some tailed amphibians, females have a higher digit ratio than males. This pattern is reversed in birds, reptiles and tailless amphibians where females exhibit lower 2D : 4D than males. These differences are probably associated with chromosomal sex-determination system. Moreover, digit ratio may vary among amphibian populations due to the susceptibility to environmental pollution. As we expect cross-species digit ratio studies could provide information on the developmental mechanism of 2D : 4D. Therefore, the main aim of this study is to investigate the 2D : 4D in the common toad *Bufo bufo* where the female is heterogametic. We hypothesized that the common toad has the similar pattern of 2D : 4D (females exhibit lower 2D : 4D than males) to other female heterogametic Anura. Moreover, as some environmental pollutants are known to have sex hormone-like effect on vertebrates, we have expected that the level of digit ratio in two different common toad breeding populations reflects varying levels of environmental pollution. We studied digit ratio in two breeding population of the common toad. The first population was located in an industrial area within the city (53 females, 52 males), while the control population was located in semi-natural habitat in the outskirts of the city (50 females, 54 males). We used computerized measuring of limb photos. In our work we want to compare digit ratio in an anuran in contrast to an urodele.

**Key words:** 2D : 4D, Anura, bioindicator, Bufonidae, sex differences
The Biodiversity-Database of the natural history museum “Haus der Natur”, Salzburg – Long term herpetological data collection as a basis for sustainable conservation

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The Biodiversity-Database of the Salzburg natural history museum (Haus der Natur) is a SQL-based database, used to collect data of wildlife- and nature-observations within the Austrian State of Salzburg. The Software Bio Office (© Tiroler Landesmuseum 2004) is the main tool used for data-input and administration. The database currently contains over 1 million observations from 15 different taxonomic groups, mostly from the region of Salzburg (~7,000 km²). About 25,000 of these records are herpetological observations from the years 1989 to 2015. The data were mostly collected as part of the work of the Herpetological workgroup of the museum (HerpAG). The observations contain data from volunteer work, scientific studies and conservational operations, as well as literature data. Almost all herpetological observations are recorded in a standardised form according to KYEK & CABELA (1996). Besides taxonomic identification and pinpoint GPS-location, they contain detailed information about the size of the habitat, habitat-type, structure and land use. The Biodiversity-Database is the backbone of practical conservation work within the region of Salzburg. It allows the precise visualisation of all observations within a specific area, as well as the analysis of ecological parameters such as distribution and habitat-usage of a species. It is furthermore planned to expand the data in a way to reliably assess specific conservation-status. The data is also made available to supra-regional exchange for neighbouring or cross-border databases (e.g. gbif). Besides being a tool for day to day conservation work the database aims to be a standardised and sustainable long term repository for biodiversity information, ensuring that this valuable data are available for future analyses. Evidence based species conservation is only possible when current, historical (and future) data are collected and stored in a standardised way!

Key words: Herpetological database, long term data collection, Salzburg, Conservation
Mitotic aberrations of germinal cells during pre-metamorphosis and their potential relationship to genome elimination in water frog hybrid

Pelophylax esculentus

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Pelophylax esculentus is the hybridogenetic species of Pelophylax lessonae and Pelophylax ridibundus. Hybridogenesis consists in elimination of one of the parental genomes from germline cells, endoreplication and clonal transmission of the remaining parental genome into gametes. Until now two putative concepts of presumably pre-meiotic genome exclusion during gonial cells proliferation phase of early gametogenesis have been proposed:
a) budding-off from interphase chromosomes or b) elimination of chromosomes during mitosis. The main aim of our research was to provide an explanation whether oogonal cells undergo proper or modified mitosis and to find relations between supposed genome rejection and potentially aberrant mitosis. By studying mitosis we will be able to demonstrate whether genome elimination takes place during mitotic phase or during the interphase. Our studies were performed on ovaries of hybrid progeny during pre-metamorphosis, in which mitotic oogonal cells are the most abundant. We applied in situ immunohistochemistry and immunofluorescence techniques on paraffin sections to identify components of the mitotic spindle. As a control we used ovaries of both parental species. The results were analyzed using light and fluorescence microscopy. During the oogenesis, apart from properly proliferating cells, atypical primary oogonia with abnormal structures and impaired mitosis, were observed in hybrids. Such cells were characterized by abnormalities in structure and shape of the mitotic spindles and presence of lagging chromosomes. Primary oogonia, characterized by enlarged diameter and double plane’s mitotic spindle and increased number of chromosomes at the same time, were noticed. Moreover, we could find both primary oogonia and diploten oocytes with double nuclei. Such research on oogonal cells of water frog hybrids have not been performed yet. Thus mechanism responsible for abnormalities described above is still unknown.

Key words: Pelophylax esculentus, aberrant mitosis, genome elimination, oogonal cells, pre-metamorphosis
Conservation implications of floods, forest management and land use for sand lizard (*Lacerta agilis*) populations

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In Slovenia the sand lizard (*Lacerta agilis*) has a distinct distribution pattern concentrated in river valleys. We have conducted an extensive field sampling using transect line visual surveys at four main rivers in the country: Sava, Drava Mura and Kolpa. Our main aims were to understand the spatial and habitat use of sand lizards on river banks and their proximity, and to analyse the effects of three major factors: floods, habitat type and forest management. Transects were located inside different flood zones (exceptional, occasional and frequent flooding) and outside the flooded area. Habitat types present at all three sites were forest, forest edge and open areas. Three river sections differed in the intensity of human-made river-bank regulations done in the past and in the current dominant land use of areas next to the river. Results showed that floods had a positive effect on the spatial distribution of the sand lizard. We propose that via exclusion of potential competitors and predators, floods could be the factor responsible for improving the conditions for sand lizards, which seemingly have an ability to survive floods in the flooding zone. Important factor further explaining the distribution in all study sites was the habitat type. Sand lizards preferentially selected edge habitats, such as forest edge or other border habitats between different vegetation. For example, at Mura river we observed a rapid population boom after forest clear-cuts, which created new (yet uninhabited) forest edges. We suggest that also forest management practices can have strong short-term effects on the population dynamics of sand lizards. Long-term effects still need to be studied.

**Key words:** *Lacerta agilis*, floods, land use, ecology, forest management
Morphological differences between *Holodactylus cornii* and *Holodactylus africanus* (Squamata: Eublepharidae)

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The genus *Holodactylus* was defined by the German herpetologist Oskar Boettger in 1893 when he described *Holodactylus africanus*, one of the two species of this genus. The second species, *Holodactylus cornii*, was described by the Italian herpetologist Giuseppe Scortecci in 1931 based on the morphological differences of four specimens. *Holodactylus cornii* was separated as a new species based on body size, number of supralabial and infralabial scales, number of scales between the nostrils and between the nostril and rostral scale. Eleven specimens of *Holodactylus cornii*, all those present in European museums, and more than 40 specimens of *Holodactylus africanus* were studied and a photo-documentation of the morphological differences of both species was made. Differences in body size, including snout–vent length, tail length and width are distinguishable features; however, they depend on the age, condition and sex of each individual. Globally it is possible to say that *Holodactylus cornii* is longer and more slender compared to *Holodactylus africanus*. Number of scales between the nostrils, between the nostril and the rostral and number of labials are the most important morphological variation.

<table>
<thead>
<tr>
<th>species</th>
<th>scales between the nostrils</th>
<th>scales between nostril and rostral</th>
<th>number of supralabials</th>
<th>number of infralabials</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. africanus</em></td>
<td>3 to 5</td>
<td>1 to 3</td>
<td>9 to 11</td>
<td>10 to 13</td>
</tr>
<tr>
<td><em>H. cornii</em></td>
<td>8 to 14</td>
<td>5 to 7</td>
<td>13 to 18</td>
<td>14 to 18</td>
</tr>
</tbody>
</table>

**Key words:** Morphology, *Holodactylus africanus*, *Holodactylus cornii*, Eublepharidae
Coastal population of Dice snakes (*Natrix tessellata*) helps clarify the evolution of euryhaline physiology in marine tetrapods

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The widespread relationship between salt excreting structures (e.g. salt glands) and marine life strongly suggests that the ability to regulate salt balance has been crucial during the transition to marine life in tetrapods. However, lacking data from intermediate stages (species lacking salt glands but occasionally using saline environments) preclude drawing a comprehensive picture of the evolution of euryhaline physiology in these organisms. Populations of Dice snakes (*Natrix tessellata*) foraging in the marine environment along the coast of the Black sea provide a unique opportunity to study physiological adaptations to marine life in a marine snake prototype lacking salt glands. We show that coastal free-ranging Dice snakes display highly variable natremia (plasma sodium concentration) ranging from normonatremia (~145 mmol.L⁻¹) up to strong hypernatremia (i.e. >195 mmol.L⁻¹). Our laboratory experiments indicate that this species can sustain life in full-strength seawater (~35 g of salt per L) up to one week with no visible long-term damage, but that such treatment entailed a marked hypernatremia that can be regulated only trough access to freshwater. Taken together these results suggest that hypernatremic Dice snakes require regular access to freshwater in order to periodically restore osmotic balance. Our results further suggest that the development of a strong physiological tolerance toward deviations of the osmotic balance (e.g., increased plasma sodium) has been a critical innovation in the evolution of euryhaline physiology and may well have preceded the evolution of salt glands.

**Key words:** salinity, natremia, marine life
A first glimpse of the phylogeography of Anatolian worm lizards

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Recently, molecular phylogenetic analyses revealed that the Anatolian worm lizard *Blanus strauchi* (Bedriaga, 1884) (Amphisbaenia: Blanidae) is actually a species complex that includes three well-defined allopatric lineages which are morphologically diagnosable and present high interspecific genetic diversity and no apparent gene-flow. Based on this evidence, the taxonomy of *B. strauchi* was re-assessed and now includes *B. strauchi*, *B. aporus* Werner, 1898 (both elevated from the subspecies to the species rank) and the newly described *B. alexandri* Sindaco, Kornilios, Sacchi & Lymberakis, 2014. In the present study, we combined published sequences with new ones, to investigate the group’s phylogeographical history. We generated a dataset of 89 specimens collected from 36 localities and used maximum likelihood and bayesian methods to reconstruct time-calibrated phylogenies. Our results show that the diversification of Anatolian *Blanus* was probably affected by the climatic changes that occurred during the Miocene to Pliocene transition, a time-period that coincides with the Messinian Salinity Crisis (MSC) of the Mediterranean. The aforementioned species are confirmed as highly differentiated clades with the addition of more samples and localities. Finally, the distribution of lineages is discussed taking into consideration the geological relief and history of Anatolia.

**Key words:** Anatolia, *Blanus*, biogeography, phylogeography, phylogeny, Turkey
Development of the tongue in the grass snake *Natrix natrix* L. (Lepidosauria, Serpentes) embryos

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The tongue of vertebrates plays a very important role in food intake. It exhibits significant morphological variations that appear to represent adaptation to the current environmental conditions of each habitat, where an animal lives. The tongue of snakes is flicked rapidly in and out of the mouth to pick up chemical molecules from the environment and transfer them to Jacobson's organ (an accessory olfactory structure). Thus, the tongue is very important in locating potential prey and enemies, in sampling food and in mating behavior. Development of the tongue in reptiles has been poorly investigated. Therefore, the purpose of this study was to describe the particular stages of the tongue development in the common species of snake in Poland – the grass snake (*Natrix natrix* L.). The histological structure of the tongue and lingual papillae in the consecutive developmental stages were studied using standard histological techniques. The age of embryos was calculated using the developmental table for this snake species. The tongue of examined species at the first developmental stage was composed of stratified squamous epithelial layer and condensing mesenchyme. At this point of development, an anterior end of the tongue was softly forked. At development stage VII the structure of the tongue resemble structure in adult specimens of this species of reptiles. The tongue was composed of a mucosa and a muscularis. The mucosa contained a stratified squamous epithelium which was non-keratinized and a tunica propria consisting of dense fibrous connective tissue. The muscularis was composed of striated muscle fibers arranged transversely, sagitally, and longitudinally. The tongue was enclosed by a sheath which contained mucous glandular tissue and striated muscle fibers. The first papillae (filiform, fungiform and cuboidalform) on the tongue surface were present at the developmental stage IX. Moreover, in the connective tissue appeared melanin cells.

**Key words:** reptiles, grass snake, tongue, development
Water frogs (*Pelophylax esculentus* complex) distribution in Belarus by using PCR-RFLP-based and DNA flow cytometry methods

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Three species of water frogs inhabit the territory of Belarus: the pool frog (*Pelophylax lessonae*), the marsh frog (*P. ridibundus*) and the edible frog (*P. esculentus*). Although *P. esculentus* complex is widespread in the territory of Belarus, this group is insufficiently studied. Almost all previous data about geographical distribution and ecology have been based on species determination by using diagnostic morphometric characteristics, established for populations from neighboring territories. The aim of our study was to estimate geographic and biotopical distribution of water frogs in Belarus. We examined 109 water bodies in Minsk, Mogilev, Brest, Grodno, Vitebsk, and Gomel regions in May-August 2011-2013. For species identification we took 1-3 individuals *P. esculentus* complex from every water body. The species affiliation was performed using PCR-RFLP-based method (175 samples) and DNA flow cytometry method (245) samples. From 109 surveyed habitats, *P. lessonae* was found in 84 (77%) water bodies, *P. esculentus* – in 47 (43%), and *P. ridibundus* in 7 reservoirs (6%). In 22 water bodies the syntopy of the edible and the pool frogs was observed. The marsh and the pool frogs were found together in two water bodies, whereas all three species co-existed in other two water bodies. *P. lessonae* was found in all types of studied habitats, as well as *P. esculentus* was found in all types of habitats, in exception of rivers, drainage channels and brooks. The rarest *P. ridibundus* was met in three groups of water bodies: oxbows of major rivers, large ponds and a drainage canal.

**Key words:** *Pelophylax esculentus* complex, distribution, Belarus, DNA flow cytometry, PCR-RFLP
The case of adenocarcinoma and cloacal mycobacteriosis in Green Tree Frog (Litoria caerulea)

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Mycobacterium fortuitum infection and adenocarcinoma were associated with cloacal prolapse in Green Tree Frog (Litoria caerulea). The disease were characterized with presence of nodules on the cloacal mucous membrane about 0.4 cm in diameter. Due to recurrent cloacal prolapse and lack of response to therapy frog was euthanized. Post-mortem examination showed no changes typical for mycobacteriosis and neoplasia in other internal organs. There was only the presence of the fragile and almost black liver and corneal lipidosis. The cloaca and other organs were examined histopatologically. Bacteriological examination of the cloacal tissue revealed presence of Enterococcus sp. and acid-fast bacteria (AFB). AFB presence was confirmed by staining cloacal tissue with Ziehl-Neelsen method and culture on the Löwenstein- Jensen medium. Species of Mycobacterium were determined by mycobacterial DNA sequencing. Cloacal swabs taken from the other three individuals residing in the same terrarium were negative for presence of Mycobacteria. Interesting issue is whether mycobacterioses and adenocarcinoma in amphibians may be linked whether their coexistence arose only from immunosuppression in that individual.

Key words: mycobacteriosis, adenocarcinoma, cloacal prolaps, green tree frog
Territoriality and homing in Alpine salamanders (Salamandra atra): a reassessment of the role of faeces for scent-marking

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Territoriality and homing are advantageous life-history traits, especially for species living at high densities, like the alpine endemic Salamandra atra. Laboratory experiments from Gautier & Miaud (2003) suggest that alpine salamanders (S. atra and S. lanzai) use faecal pellets as marks for shelter recognition, playing a putative role in both homing and territorial behavior. Adult individuals avoided shelters with faecal pellets from conspecifics, while they were attracted by their own faeces. Furthermore, juvenile faeces did not affect the behavior of conspecifics, but juveniles were attracted by faeces from adults. In contrast to these findings, it is fairly common to observe several individuals under the same shelter in the field. Building on these facts, we assume 1) that the status of an individual (juvenile, male, female) can be recognized based on the scent marks (volatile organic compounds) and 2) that the strength of territoriality depends on the relatedness between the individuals: closely related individuals putatively have similar scent marks and thus will be tolerated under the same shelter while unrelated individuals will be excluded. To test these hypotheses, we sampled faeces of 90 individuals coming from three different sites (site of origin used as a proxy for the relatedness) in the state of Salzburg (Austria). Behavioral experiments were performed by adjusting the design described by Gautier and coworkers (Gautier & Miaud 2003; Gautier et. al., 2004) and scent-marks were analyzed by gas chromatography coupled to mass spectrometry (GC-MS) to determine the compounds which may play a role for individual recognition and for the assessment of conspecific characteristics (age, sex, relatedness). Here we present the results of the scents analysis and discuss them in the light of our behavioral experiments.

Key words: Amphibian faeces, behavioral experiment, gas chromatography-mass spectrometry, volatile organic compounds
Nesting and spatial ecology of Emys orbicularis in the Ljubljana Moors, Slovenia

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The European pond turtle (Emys orbicularis) is widely distributed in Slovenia, with bigger populations in the Ljubljana Moors, Bela krajina, Sava river basin and at the Slovene coast. Currently there is insufficient knowledge about spatial ecology reproductive success of Emys orbicularis in Slovenia. This kind of information is crucial for establishing effective conservation management strategy. E. orbicularis is one of the species with the highest conservation status among reptiles under national and European legislation and considered endangered by the national red list. We conducted our study in the Ljubljana Moors were a bigger population of Emys orbucularis is present, but no juvenile terrapins were found in previous years, which could be due to unsuccessful reproduction. Here E. orbicularis occurs in melioration channels, which are surrounded with agricultural landscape; meadows, corn and wheat fields. Most probably females lay their eggs at sites, which are disturbed by farming works and destroyed before hatchling. The main aim of our study was to find nesting sites and protect them from predators and agricultural machinery work in order to ensure a successful reproduction. Secondary, we aimed to define movements and home ranges of marked individual and to find out how often they cross roads, where they can be ran over. To answer these questions we equipped all together ten (7 females and 3 males) individuals of E. orbicularis with radio transmitters. Using telemetry, we tracked them every night in the period when females were about to lay the eggs (from mid-May to mid-June). After that, we determined their position once every week. All nests found were protected with a mesh-wire and will be monitored frequently in the time of hatchling (expected in late August and September) to prevent destruction by farming works and ensure successful reproduction of E. orbicularis in the Ljubljana Moors.

Key words: Emys orbicularis, nesting site, home range

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Comparison between mating calls of *Hyla arborea* (L., 1758) and *H. orientalis* Bedriaga, 1890 from Bulgaria

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It was recently established that most tree frog populations on the territory of Bulgaria, previously thought to be *Hyla arborea*, belong to the *H. orientalis* taxon. According to molecular data, *H. arborea* is present in Struma river basin. We recorded mating calls of both species from three localities – *H. arborea* form South Struma valley (Hursovo), *H. orientalis* from southern Black Sea coast (Silistar) and central Trakia plain (Shishmantzi). Calls were tested for differences between their spectral and temporal characteristics. The following parameters were used in the analyses: number of pulse groups (PG), number of pulses per PG, PG duration, PG interval and dominant frequency. We used a Kruskal-Wallis test with locality and species as grouping variables. Results demonstrated that while there was no statistically significant difference between calls from the two populations of *H. orientalis*, both were significantly different to calls of *H. arborea* in respect to number of pulses per PG and number of PG (p<0.01). For *H. arborea* both the number of PG and the number of pulses per PG were lower. No significant differences were observed in the other studied parameters. Since all recorded specimens called in the water and water temperature was similar (18-20°C) in all three localities, it could be hypothesized that *H. orientalis* could potentially emit longer calls with more pulse groups and more pulses per PG. In our view, the number of pulses per PG is of greater significance for differentiating between the two species, because the number of PG could be influenced by the individual’s condition and various environmental factors. However, more research is needed to establish whether these species could be differentiated based on their calls. For now it seems unlikely that call parameters are very important for preventing interspecific hybridization.

**Key words:** bioacoustics, call parameters, differences, mating call, tree frogs
Breeding ecology and active conservation of yellow-bellied toad
*Bombina variegata* – a case study at the Biała river

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The main objective of the project was to restore the continuity of the habitats between two separated populations of a yellow-bellied toad, along the Biała River. The project assumed to set up a series of stepping stones along the river, connecting two populations of amphibians. At each stepping stone a pair of the water bodies was constructed, managed in different way: one was cleared from all water vegetation and predatory invertebrates, while the other was left without interference, allowing for spontaneous succession. 100 yellow-bellied toad tadpoles, taken from places threatened with destruction, was introduced to each of the waterbodies in order to check the influence of the succession on young production. Tadpoles and individuals after metamorphosis were counted systematically in each pond. Reproductive success in the cleaned waterbodies was: 24.85% (season I) and 28.9% (season II), while in waterbodies with a succession the reproductive success was: 19.46% (season I) and 11.4% (season II). It follows that the success of breeding yellow-bellied toads depends on the predatory pressure of invertebrates in the water bodies. This was also confirmed by our research on emergence phenology of predatory invertebrates and also by the tadpoles occurrence in reference to the moment of invertebrates egg laying. There are no bugs and beetles in aquatic habitat during the period of egg laying of yellow-bellied toads. Later in the season many more larvae of dragonflies and predatory beetles occur. Also the juveniles dispersion from the water bodies was analysed. It was found that the main direction of migration was the riverbed, where toads remained in the shallows, rocky banks or at the clay shore. Telemetry studies of adults in diverse habitat environment were also conducted. It was found that most of the individuals released within the floodplain disperse to the river channel, thus, it plays an important role as a migration corridor for yellow-bellied toads. The study was financed by EU project POIS-05.02.00-00-084 / 08.

**Key words:** *Bombina variegata*, breeding ecology, stepping stone habitats, reproductive success, predation pressure, telemetry
Preliminary results from observation of blood-sucking flies genus *Sycorax* (Diptera: Psychodidae) hosted by *Ansonia longidigita* (Anura: Bufonidae) in Borneo

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Flies are one of the most common nonlethal parasites found in frogs and toads. Detailed knowledge about relations between frogs and ectoparasitic flies are inadequate. Our study was conducted in the lowland mixed-dipterocarp rainforest in the Ulu Temburong National Park, Brunei Darussalam (04.546°N, 115.157°E). During the field studies, we recorded 24 individuals of *Ansonia longidigita* (22M, 2F), and on seven males found 93 flies of *Sycorax* (90M, 3F). Flies were collected using a sucking aspirator, preserved in 96 % ethanol and one female specimen was dissected. All specimens of *Sycorax* were found in contact with throat, dorsal surface of body, and extremities of male individuals of *Ansonia*. The flies were found during the night, when toads are active (climbing on rocks or vegetation) adjacent to the study stream. The flies apparently use calling male frogs as lek arenas where they display, or show scramble competition, to gain access to females. It is known that only *Sycorax* females bite and suck blood, because only they have mandibles. Our findings confirm this as amphibian blood was found in dissected female fly. The high number of male flies collected on *Ansonia longidigita* can be interpreted as aggregation behaviour, with males probably attracted by the frog’s advertisement calls. Collected flies are being described as a new species *Sycorax konopiki* sp. nov.

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**Key words:** parasitism, blood sucking, *Sycorax*, *Ansonia*, Ulu Temburong NP, Brunei
Two tree frog lineages (Hylidae) in Poland: distribution and hybrid zone localization

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The application of genetic markers has resulted in a partial revision of the systematics of Western Palearctic tree frogs (Stöck et al., Mol. Phylogen. Evol. 2012). Currently, 10 taxa are recognized: Hyla arborea, H. molleri, H. felixarabica, H. intermedia, H. meridionalis, H. orientalis, H. sarda and H. savignyi; two of them (one related to H. meridionalis, another to H. intermedia) still have not been formally described. Studies carried out in central and northern Poland (Dufresnes, Majtyka et al., submitted) revealed a parapatric distribution of two species: H. arborea and H. orientalis. Hyla arborea inhabits western Poland, up to the Baltic Sea, and reaching the southern moraine hills of the Lake Districts. The eastern taxon, H. orientalis, occurs east of the Vistula river, in some places traversing it to the West. Both species form a hybrid zone across central Poland. Populations of H. arborea from the study area have a significantly lower expected heterozygosity and slightly lower allelic richness, compared with populations of H. orientalis and hybrids. This indicates less genetic diversity of H. arborea. In contrast, H. orientalis exhibits a greater genetic diversity. High FST within H. arborea indicates a high genetic differentiation among populations of this species, while FST of H. orientalis is very low, which indicates a lack of differentiation among its populations. This indicates a larger contemporary gene flow between H. orientalis populations compared with those of H. arborea.

Key words: hybrid zone, Hyla arborea, Hyla orientalis, Poland
Diet of wild populations of *Testudo hermanni* in Italy: implications for the management of confiscated individuals

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*Testudo hermanni* is a tortoises distributed in Southern Europe. In Italy this species is mostly found throughout the Peninsula, Sardinia and Sicily, occurring mainly in Mediterranean coastal and hilly natural/semi-natural habitats. It is an herbivorous species but its diet can also include mosses, mushrooms, carrions, small invertebrates, excrements as well as bones. It is important to know in depth the diet of the different wild populations in order to properly manage the confiscated individuals, to plan their correct relocation in the wild as well as to preserve their habitats. With this aim, in March-July 2014 we analyzed the diet and the foraging behavior of some Italian wild populations (Apulia, Tuscany and Sardinia). Fecal samples were collected and the content analyzed by micro-histological techniques. Our results indicate that several plant families compose the diet of this species; The most actively selected in all the surveyed areas are the Fabaceae (*Trifolium*, *Medicago*, *Vicia*). Other detected ones are: Plantaginaceae (*Plantago*), Rubiaceae (*Rubia*), Smilaceae (*Smilax*), Caprifoliaceae (*Knautia*), Poaceae (*Melica, Stipa, Vulpia*), Brassicaceae (*Lobularia*), Rosaceae (*Rubus*), Ericaceae (*Arbutus*), Oleaceae (*Phillyrea*), Fagaceae (*Quercus*), Asteraceae, Araliaceae, Lamiaceae (*Teucrium*) and Cistaceae (*Cistus*). Despite their abundance in all the study areas, Poaceae were little selected. Seeds of *Knautia integrifolia* and fruits of *Phillyrea angustifolia* were also found in some fecal samples. In addition, mushrooms, mosses and gastropods’ shell fragments were observed. The dominance of Fabaceae is seemingly related to their high protein and calcium content. The presence of seeds and fruits support the hypothesis that terrestrial tortoises play an important role as seed dispersers.

**Key words:** *Testudo hermanni*, diet, wild populations, relocation, microhistological techniques
Comparison of metabolic rates in two competing lacertid species (*Podarcis muralis* and *Iberolacerta horvathi*) across an altitudinal gradient

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Among the important physiological factors having an influence on the survival of ectothermic organisms, metabolism is a rarely studied in lacertid lizards. Common wall lizard (*Podarcis muralis*) and Horvath’s rock lizard (*Iberolacerta horvathi*) exhibit a partial altitudinal segregation pattern with *P. muralis* more abundant at low altitudes, while *I. horvathi* prevails at higher altitudes. Both species ranges overlap at middle altitudes where in syntopic populations they are likely in competition. In this study we compared metabolic rates between *P. muralis* and *I. horvathi* from populations at three different altitudes (low, middle, high). Our previous research has already shown that lizards of both species from similar mid-altitudes have similar metabolic rates. Since metabolic rate in ectotherms is temperature dependent, the most obvious environmental trait which drops with an increase of altitude, we predict for a given species higher metabolic rates in populations at higher altitudes than from lowlands. Metabolic rates were obtained by measuring of oxygen consumption from each species-altitude population. Furthermore, we tested for the response of metabolic rates at different temperature regimes, namely, 23°C, 28°C and 33°C. We tested two alternative hypotheses. If results will show a parallel change in metabolic rates across the altitude in both species, this means that both respond to environmental changes in temperature in the same manner. If one species will show an increase of metabolic rates in populations at high altitudes, this might present an advantage of surviving in environments with more restrictive environmental temperatures, and would present a competitive advantage in this species pair in interaction.

**Key words:** metabolic rate, temperature, interspecific competition, *Podarcis muralis, Iberolacerta horvathi*
Does location of Common Toad breeding ponds in urbanization gradient influence individuals’ body condition?

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It seems that increasing urbanization, and related habitat loss is the greatest thread to amphibians. The aim of the study was to find whether location of breeding pond in urbanization gradient influence condition of Common Toads *Bufo bufo*. The study was carried out in Warsaw (Central Poland) during toad breeding season (mostly April) 2011-2014. Breeding adults were caught, weighted and measured (body length) in 6 ponds differ in urbanization pressure (distance to roads and buildings, character of shore line, etc). Due to small number of females, final analyses were carried out only on males. Condition of individuals was described using Body Condition Index (BCI), obtained from residuals of body mass and body length regression.

It was found that breeding pond location significantly influenced condition of toad males, however, contrary to expectations, individuals from ponds located in areas with intermediate to high urbanization pressure were in general in better condition than individuals from outskirts of the city, and natural reserves. It was found that “year” variable not influenced obtained results, but interaction location×year was significant, suggesting that condition of breeding individuals changed differently between years in particular ponds, probably due to different wintering conditions provided by habitats surrounding those ponds.

The study was financed by MNiSW/NCN grant no N N304 335839.

**Key words:** *Bufo bufo*, Common Toad, amphibians, urbanization, body condition
Ecological differentiation among Moroccan populations of the Mediterranean Pond turtle *Mauremys leprosa*: an environmental niche modeling

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Spatial distribution of taxa can be predicted using Ecological Niche Modeling (ENM). This method based on spatially-explicit environmental information and geo-referenced data, provides an estimate of the ecological niche; the multi-dimensional environmental space within which a species can persist. ENM can be effectively used to assess lineage delimitation, showing great utility in groups that exhibit low levels of molecular differentiation and little morphological divergence. By performing a climate niche analysis and developing ENM based on confirmed records of six putative subspecies of the Stripe-necked turtle, *Mauremys leprosa*, from Morocco we aimed at: (i) assessing whether they inhabit different climatic niches, (ii) assessing whether they fulfill the required conditions to be considered different incipient evolving lineages under low genetic and morphological differentiation. On the basis of 156 geo-referenced specimens, we developed ecological niche models using Maxent programme and spatially explicit climate data to examine historical and ecological factors affecting variation in *M. leprosa*, across its range in Morocco. Overall, we found little support for the recognized subspecies as either independent evolutionary lineages or geographically circumscribed units and conclude that although some genetic and niche differentiation has occurred, most populations assigned to *M. leprosa* appear to represent a single, widespread species. However, additional sampling and application of nuclear markers are necessary to clarify the status of the southernmost populations. Even climatic niche differentiation among populations was detected, only two subspecies were revealed by climate niche analyses and ecological niche models. However, each population should be considered as a unit for conservation and management.

**Key words:** Ecological Niche Modeling, geo-reference, climatic niche, Maxent, *Mauremys leprosa*, Morocco
Genetic structure of *Salamandra salamandra* at the northern margin of its range in the Carpathians

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Amphibian populations inhabiting the margin of the species range are often exposed to adverse genetic processes expressed by their higher genetic differentiation rate and isolation. Inability for long migrations, breeding site fidelity, particular environmental preferences together with multiple anthropogenic activities and habitat changes may cause reduction of effective population size and accelerate processes leading to local extinction. In this study, we have investigated genetic variation and structure of the fire salamander *Salamandra salamandra* inhabiting northeastern border of the species range in Polish part of the Carpathian Mountains. Gene flow and possible differentiation among 11 locations were measured using polymorphism of 10 nuclear microsatellite loci in 380 individuals. Mitochondrial DNA control region among 17 individuals selected from 13 localities was sequenced to trace the origin and recolonization pattern after the last glacial cycle. Generally, in most of the studied Carpathian populations, analysis such as pairwise population F\(_{ST}\) or AMOVA revealed relatively high level of genetic variation and little differences without evidence of strong barriers to gene exchange. However, two populations were characterized by reduced variation, thus placed under the strong influence of genetic drift. All analysis clearly distinguished one extreme marginal, isolated and highly differentiated population from the Carpathian Piedmont. Bayesian analysis in STRUCTURE also showed subtle structuring between eastern and western part of the studied region. Mitochondrial DNA homogeneity among all individuals represented by single haplotype suggested that *S. salamandra* recolonized northeastern part of Europe from glacial refugium located in the Balkan Peninsula.

**Key words:** gene flow, genetic structure, isolation, microsatellites, mtDNA, *Salamandra salamandra*
Significance of reptile surveys conducted at biology student research camps for distributional data of Reptilia in Slovenia

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Slovenia is home to all together 21 species of reptiles (excluding sea turtles); 9 lizards, 11 snakes and one freshwater turtle, *Emys orbicularis*. The distribution of most of the species is still not completely known mostly due to the lack of any national program to survey or perform monitoring of reptile populations. However, the knowledge on species distribution shows a trend of improvement mostly because of volunteer work of the Slovene Herpetological society – Societas herpetologica slovenica members. One of the forms of systematic reptile surveys is done on a yearly basis when biology students from Biotechnical faculty of University of Ljubljana organize research camps at different locations around the country. We have analyzed the distributional data of reptiles obtained at 13 different camps in the period of 2002-2014. Our aim was to evaluate the significance of these surveys for the knowledge on distribution of reptiles on the national level. We used a 10×10 km UTM grid of Slovenia, with the number of species per square in the database before 2002 and after each student camp to obtain the number of new species recorded for each surveyed square. Each year there were new records for at least one or more UTM squares and even up to 8 new reptile species were found for a UTM square. In total, records gathered at research camps contributed to better species distribution maps on the national level for at least 17 species. Overall, around 50% of the country surface was covered and all data was inserted in the national reptile data-base held by society and Center for cartography of fauna and flora. Thus, we conclude that such volunteer-based surveys have a high importance for the state of the national reptile database in Slovenia today.

**Key words:** reptiles, species distribuiton, Slovenia
Using Geometric Morphometrics to assess head shape variation in *Vipera seoanei*

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The head shape of snakes has a major role in their ecology and usually exhibits morphological variation as a response to natural and sexual selection. Here we aim to describe how head shape varies in the Iberian viper, *Vipera seoanei*, in relation to growth, sex and color phenotype. *V. seoanei* is a European viper almost endemic to the northern Iberian Peninsula, with five color morphs, but low genetic diversity. We used geometric morphometrics on 147 specimens from its distributional range, representing different ages, sexes and color morphs. We quantified head shape by digitizing 20 landmarks on the dorsal side of the head. We then superimposed landmark coordinates to remove the effects of location, scale and rotation and obtain shape variables, based on which we performed standard multivariate analyses to explore patterns of variation. Principal Component Analysis showed that the first component of shape variation (49.47% of total variance) was associated to changes in the posterior region of the head and it represented a contrast between vipers with a short but wide posterior head. Shape variation related to the second principal component (25.77% of total variance) represented a contrast between vipers with a narrow posterior head and a wider snout. MANOVA comparisons yielded no significant differences among sexes or color morphs. However, head shape was significantly different between vipers of different ages. Head shape varied allometrically with head size, under a common slope in all groups, where head growth was associated to an enlargement of the posterior region of the head and a lateral reduction of the area of the eyes. This study provides a methodological framework for the implementation of geometric morphometrics for the study of head shape variation in European vipers, which will be useful to explore other sources of variability, such as environmental and ecological factors.

**Key words:** sexual dimorphism; ontogeny; allometry; phenotypic variability; Iberian Peninsula
Comparative age dimorphism in three members of the genus *Lacerta* (Reptilia: Squamata: Lacertidae)

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Ontogenetic changes in morphology have received little attention, with the so-called age dimorphism being studied substantially less than the largely recognized sexual dimorphism. Three taxa having spatially parapatric distribution in the Eastern Balkan Peninsula were chosen for this study, namely: *Lacerta agilis bosnica*, *L. a. chersonensis* and *L. viridis*. The first taxon is confined to mountainous climatic regions, the second one typically inhabits meadows in medium altitudes and peri-mountainous plains in the temperate zone, and the third is more thermophilic, widespread across the study region. A set of commonly stated measurements (SVL, pileus length / width, head width and extremities length) was taken and expressed as proportions against SVL. In order to compare the level of differentiation among juvenile and mature specimens within and in between taxa mahalanobis distances were calculated via canonical discriminant analysis. Analysis of variance (ANOVA) was also performed. Sexes were treated separately. In the sand lizard subspecies age dimorphism was reversely displayed but with similar values – greater in female *L. a. chersonensis* and respectively in male *L. a. bosnica*. In *L. viridis* distance values were highest amongst the three taxa, being greater in males. During the growth process the pileus length retained its greatest values in males *L. viridis* and decreased in order in *L. a. chersonensis* and *L. a. bosnica*, while pileus and head width retained similar values among taxa after maturation. No differences among age classes in respect to front leg length were found but to the length of the hind legs in all females decreased in time. Future research on external and internal driving forces that influence the morphological variation during the growth process should be performed in order to clarify their interrelationships.

Key words: Lacertids, growth, morphology, ontogenetic changes
Estimating the body condition of amphibian populations is important because it may reflect habitat quality. Body condition index can be calculated from routinely collected length and weight data of individuals. The aim of this study is to determine body condition for *Salamandra salamandra* population in a landscape heavily influenced by human activities. The extensive alterations at Pokupsko basin has resulted in changes in wetland hydrology and a probable shift in the relative abundance of amphibian species. Condition indices of the fire salamander population were estimated over a 3 year period capture-mark-recapture study in an area of 4,25 ha in the temperate forest in central Croatia, where 4 permanent plots were installed. This study was based on body measurements for 90 adults and juveniles from the fire salamander population. I compared the residuals from ordinary least squared regressions of salamander weight to total length as an index of body condition. A Kruskal-Wallis one-way ANOVA was used to test for differences in body condition among permanent plots and among seasons during the study. Results indicated significant differences in residuals between the two plots (\(K = 3.92, \text{df} =1, p = 0.05\)). Salamanders with positive residuals and thus better body condition were captured in the plot situated in the forested area with better habitat conditions (e.g. proximity of forested pond). Season had no significant effect on residual index (\(K = 0.001, \text{df} =1, p = 0.05\)), but the test showed that residuals were positive in spring and negative in autumn. The residual index is a useful tool in monitoring and conservation of amphibian populations because it is correlated with habitat quality and can be used to interpret the effects of environmental factors.

**Key words:** body condition index, *Salamandra salamandra*, forest disturbance, conservation
Detection of the European pond turtle using environmental DNA from water samples – first results

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Environmental DNA (eDNA) is a recent molecular technique used to detect species in natural habitats. eDNA breaks down in water within 1-4 weeks, so consequently the detection of species’ DNA in water confirms its recent presence. In this study, we developed a method allowing detection of *Emys orbicularis* in water samples using qPCR technique. Water samples (1 l) from known locations of *Emys orbicularis* (5 in Poland and 2 in Lithuania) were collected in early spring 2015 to sterile bottles. Four membrane (mixed cellulose ester) filters were processed for each sample. DNA was extracted with PowerWater DNA Isolation Kit (Mo Bio). The four extractions from each site were next analyzed separately by qPCR technique. Primer pair and TaqMan probe were designed using IDT PrimerQuest from own cytb gene sequence data of *Emys orbicularis* and sequences available in GenBank. All designed primer pairs and probes were blasted against the NCBI nucleotide database, and the assay specific to target species but not to co-occurring taxa was chosen. First, we performed analysis of serial dilutions of DNA extracted from buccal swabs collected from the target species (from 300 ng to 16 pg). Next, we tested three PCR replicates from each extraction in presence of NTC and PTC. DNA Extraction Control (DEC 560, Bioline) was also used to monitor effectiveness of the extraction and co-purification of PCR inhibitors. In majority of the samples analyzed we obtained positive results (from 1 to 3 positives per filter). We observed inhibition in all four filters (much lower fluorescence of DEC) in two samples, out of which we had all negative results for the first sample and one positive repetition for the second one. The preliminary results obtained are promising, however in further research, selected methodological issues will be analyzed in order to optimize the method.

**Key words:** *Emys orbicularis*, eDNA, species detection, conservation genetic
Northern Fire-Bellied Toad *Bombina bombina* as possible Indicator species for climate change: the expansion of its distribution since 1929 and its conservation issues in Latvia on the northern edge of its European range

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*Bombina bombina* (Linnaeus, 1761) inhabits in Latvia on the extreme northern edge of its European range. Since 1929, it was known only about one population “Bauska” in Latvia with ~10 vocalizing males. The second population “Ilgas” with the same number of males was discovered in 70’s only. By carrying out the search for new populations until 2015 and total monitoring of all populations in 2004 – 2011, we have noted an increase in number of the populations and localities, the expansion of the range of the populations and their merger into metapopulations. Thus, in the final year of the total monitoring we noted already 9 existing populations of *B. bombina* with 22 new localities and 105 vocalizing males. The possible reasons for this may be the decrease of the agriculture in Latvia; increase of the number of beavers *Castor fiber* and the destruction by the beavers the melioration systems; successful implementation of the *B. bombina* protection plan; as well as the climate change in the region. Thus, the average annual air temperature and the amount of precipitation have been exhibiting a strongly pronounced upward trend in Latvia, including the *B. bombina* range, since the 1950s. However, all the known populations are located not further than 20 km from the southern boundary of Latvia. The major man-induced threats to *B. bombina*, noted in the research, are the draining of ponds; water and sound pollution; the destruction by the people the beaver-made dams and extermination of beavers; the distribution by the people different species of fishes, including allochthonous invasive Amur sleeper *Percottus glenii* (Dybowski, 1877). The parts of the research were supported by project "Creation of a new scientific group for modernization of aquaculture technology" # 2013/0067/1DP/1.1.1.2.0/13/APIA/VIAA/060; and by the Project LIFE-HerpetoLatvia. Some conservation measures are suggested in the conclusion.

**Key words:** *Bombina bombina*, distribution, Latvia, nature conservation
Inventory and Monitoring Herpetofauna in Ujung Kulon National Park, Indonesia

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Herpetofauna is one of the existing diversity in Indonesia and in this aspect of research still be animals that lack of attention. Ujung Kulon National Park have ideal habitat for herpetofauna because the type of forest is quite diverse. Inventory and monitoring is done every year to see the development of these animals. The method used is Visual Encounter Survey (VES) that making a direct encounter species in terrestrial and aquatic areas. The results of the inventory are obtained 19 species with the details as much as 9 species of amphibians, while reptiles as many as 10 species. Comparison over the last three years shows that there are fluctuations in the data caused by several factors, ranging from the number of observers, the ability of the observer, or different weather for the last three years.

**Key words:** fluctuations, herpetofauna, Ujung Kulon National Park
Effects of dietary probiotics on growth performance and gastrointestinal tract microbiota of Florida softshell turtle (Apalone ferox)

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Softshell turtle farming for the food market is a growing branch of aquaculture in Asia. In the farming environment turtles are exposed to many pathogens and pose a threat to people who come into contact with them. Improvement in growth performance and product safety may additionally be a reason for a decrease in environmental pollution and constitute an alternative source of turtle meat to wild, extinction-threatened and overharvested populations. The above mentioned facts are the reasons for studying turtle nutrition, in order to decrease zoonothical threats connected with their microbiota and to improve growth performance parameters in turtles. The aim of the study was to assess the effects of dietary probiotics on growth performance, condition, development and microflora of gastrointestinal tract in Florida softshell turtles (Apalone ferox). The experiment was performed on 30 young Florida softshell turtles (Apalone ferox) divided into three groups. Animals were kept individually and fed on experimental diets: without additives (CON), with addition of Bacillus subtilis PB6 (SSP) or multiple strain probiotic (MSP) including Lactobacillus, Bifidobacterium, Streptococcus, Enterococcus, Aspergillus and Candida (MSP). During the experimental period growth performance parameters were measured. In the 52nd week of the experiment the animals were euthanized and morphometric analyses of gastrointestinal tract were performed. Selected groups of bacteria excreted to water were enumerated on various types of selective agars. The diet supplemented with SSP positively affected growth performance of turtles. The applied MSP increased the ratio of gastrointestinal tract length to carapace length, and decreased the number of bacteria excreted to water. It was also recorded that the number of bacteria resistant to kanamycin and vancomycin was increased in case of SSP treatment compared to MSP.

Key words: Apalone ferox, probiotics, turtle, gastrointestinal tract microbiota
Effects of dietary probiotics on growth performance and gastrointestinal tract microbiota of common musk turtle (*Sternotherus odoratus*)

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Turtle farming is becoming more and more important part of aquaculture. The animals are bred for the Asian food market and pet industry worldwide. However, they continuously pose a microbiological threat related with the presence of many potentially pathogenic bacterial strains in the reptile gastrointestinal tract and in the environment. Moreover, turtle breeding and rearing is an important tool of active species protection, as it reduces the time young turtles need to reach the size that makes them less likely to fall victims of predators after reintroduction. These facts are the reasons for studying turtle nutrition, in order to decrease zoonothical threats connected with their microbiota and to improve their growth performance parameters. The aim of the study was to assess the effects of dietary probiotics supplementation on growth performance, condition, development and microflora of gastrointestinal tract in common musk turtles (*Sternotherus odoratus*). The experiment was performed on 30 young common musk turtles (*Sternotherus odoratus*) divided into three groups. The animals were kept individually and fed on experimental diets: without additives (CON), with addition of *Bacillus subtilis* PB6 (SSP) or multiple strain probiotic including *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, *Aspergillus* and *Candida* (MSP). During the experimental period growth performance parameters were measured. Selected groups of bacteria excreted to water were enumerated on selective agars. In the 52nd week of the experiment the animals were euthanized for morphometric analyses of gastrointestinal tract, sample collection for FISH and histological analyses. Diet supplementation with MSP positively affected growth performance of turtles, increasing body weight gain and decreasing feed conversion ratio (P<0.05). SSP addition caused lower total aerobic, anaerobic, haemolytic, kanamycin and vankomycin bacteria counts, as well as *Enterobacteriaceae*, *Clostridium difficile* and *Staphylococcus* sp. concentrations in water. Both probiotics caused decreased numbers of total bacteria, *Enterobacteriaceae*, *Clostridiaceae* and *Bacteroides* (P<0.05).

**Key words:** *Sternotherus odoratus*, probiotics, turtle, gastrointestinal tract microbiota
Genetic divergence in tropical anurans: deeper phylogeographic structure in forest specialists and in topographically complex regions

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Many tropical organisms show large genetic differences among populations, yet the prevalent drivers of the underlying divergence processes are incompletely understood. We explored the effect of several habitat and natural history features (body size, macrohabitat, microhabitat, reproduction site, climatic heterogeneity, and topography) on population genetic divergence in tropical amphibians, based on a data set of 2680 DNA sequences of the mitochondrial cytochrome b gene in 39 widely distributed frog species from Brazil, Central America, Cuba, and Madagascar. Generalized linear models were implemented in an information-theoretic framework to evaluate the effect of the six predictors on genetic divergence among populations, measured as spatially corrected pairwise distances. Results indicate that topographic complexity and macrohabitat preferences have a strong effect on population divergence with species specialized to forest habitat and/or from topographically complex regions showing higher phylogeographic structure. This relationship changed after accounting for phylogenetic relatedness among taxa rendering macrohabitat preferences as the most important feature shaping genetic divergence. The remaining predictors showed negligible effects on the observed genetic divergence. A similar analysis performed using the population-scaled mutation rate (Θ) as response variable, showed little effect of the predictor variables. Our results demonstrate greater evolutionary independence among populations of anurans from forested regions versus species from open habitats. This pattern may result from lower vagility and stringency in reproductive requirements of rainforest species. Conversely, open landscapes may offer ephemeral and unstable breeding sites suitable for vagile generalist species, resulting in reduced intraspecific divergence. Our results predict that, for a given period of time, there should be a higher chance of speciation in tropical anurans living in forests than in species adapted to open habitats.

Key words: Amphibia; Anura; phylogeography; cytochrome b; geographic distance; population divergence; Madagascar; Brazil; Cuba; Central America
Gametogenesis and development of gonads in diploid and triploid water frogs
(*Pelophylax esculentus* complex)

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Water frogs are diploid or triploid bisexual hybrids with a special way of gametogenesis, during which one of the parental genomes is eliminated and the resulting gametes are clonal (hybridogenesis). The genome elimination occurs during a prolonged period of gonial proliferation, and meiosis starts when one set of chromosomes is properly rejected.

The gonads in hybrids, both diploid and triploid, display delayed development and various malformations. The cortex of hybrid ovaries is composed of dividing primary oogonia, among which 15–75% degenerate. The onset of meiosis is delayed about 1 year and is prolonged in comparison to the parental species (*P. lessonae* and *P. ridibundus*). The development and differentiation of testes in hybrids are also delayed and seminiferous tubules contain fewer germ cells. The mitotic activity of primary spermatogonia is high in the hybrids, but most of the resulting germ cells degenerate. Adults are often less fertile and have abnormal gonads. In such testes degeneration of all stages of spermatogenesis is very high, spermatozoa are scanty, and seminiferous tubules are abnormally organized. Gonad differentiation in triploids is faster than in diploids; this may be caused by a way of genome elimination before the onset of meiosis: in diploids one of the genome is eliminated and the remaining one is duplicated, whereas in triploids also one genome is eliminated, but two remaining ones (belonging to the same parental species) are ready to enter meiosis and do not need reduplication.

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**Key words:** hybridogenesis, gametogenesis, gonad development, polyploidy
Distribution of amphibians and reptiles in Tatra National Park (southern Poland)

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Amphibians and reptiles are declining at a global scale. Protected areas, such as national parks, provide a unique opportunity to preserve the entire habitats of the herpetofauna, however they are not free from negative human impact. Hence long term monitoring and occasional field inventories have particular importance. Our goal was to describe the current distributions of amphibians and reptiles in Tatra National Park (TPN) in southern Poland and to compare our observations with historical data. Field work was carried out during two seasons (2013–2014) and covered nearly all of the area of the montane and subalpine zones (800–1906 m.a.s.l.) along the valleys of TPN. Six amphibian species (Salamandra salamandra, Lissotriton montandoni, Ichthyosaura (Mesotriton) alpestris, Rana temporaria, Bufo bufo, Bombina variegata) and 2 species of reptiles (Zootoca vivipara, Vipera berus) were found. A significant decline in the number of breeding localities was found for 4 amphibian species (S. salamandra, L. montandoni, I. alpestris, B. variegata) in comparison to historical data. These declines were driven by both loss of breeding sites as well as local extinctions despite the persistence of intact breeding habitat. The greatest decline concerned S. salamandra (from 10 to only 1 locality) and B. variegata (from 22 to 7 localities). Both species of reptiles, Z. vivipara and V. berus, are still widely distributed and numerous in TPN. Other species of reptiles, historically reported, were not detected (Lacerta agilis, Anguis fragilis/colchicus, Natrix natrix). The most important threats in TPN include an increase in tourism, restocking of fish and natural succession in ponds, overgrowing of glades and meadows, and road mortality.

Key words: amphibians, reptiles, Tatra Mountains, monitoring, distribution
The future of the European pond turtle (*Emys orbicularis*) in the Czech Republic

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*Emys orbicularis* is one of the most endangered native species in the Czech Republic (CR) and its occurrence is gradually decreasing. The reasons for this are draining of wetlands and ponds, transformation of the land to fields or forests, and also hunting for meat. Turtles also served for lenten meals and since the Middle-Ages have been imported from more southerly countries. At present, non-native populations and individuals in CR have been recorded namely in the regions with warmer climate. Successful reproduction occurs in the locality of Betlém (southern Moravia), where the total number of specimens is estimated to 200. However, survival of native turtles cannot be excluded. Data from BioLib (www.biolib.cz) and from the Species Occurrence Database of the Nature Conservation (http://portal.nature.cz) obtained thanks to the participation of both professional and lay public show current incidence of *E. orbicularis* in some localities and provide hints for searching for the native turtles.

Organization HERPETA, in collaboration with the experts from the Nature Conservation Agency of the Czech Republic, some universities, museums, and zoological gardens launched in 2014 a long-term project “The European pond turtle in the Czech Republic“. The project is aimed at preservation of the last *E. orbicularis* and their return to the localities where they are extinct. The initial steps of the project consist in collecting data on the current occurrence, assessment of the habitat and temperature characteristics of selected localities (namely potential suitability for successful incubation), and identification of affiliation of the turtles to phylogenetic lineages. *E. orbicularis* is a suitable umbrella species; restoration of sandbanks would create stations for other rare animal and plant species. Further going on is monitoring of the occurrence and effect of non-native turtle species, which represent competition for the *E. orbicularis* and potential risk of infection spread to other animals as well.

**Key words:** conservation, *Emys orbicularis*, native turtles, non-native turtles, reproduction, umbrella species
iButtons as a useful device to record the operative environmental temperatures for small lizards

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Operative environmental temperature (Te) is a measure that describes the null distribution of body temperature (Tb) expected in non-regulating animals. This metric has been developed to assess whether an individual is actively thermoregulating. Te is the product of interactions between biophysical and morphological factors that influence the Tb of an inanimate object similar in size, shape and colour to the study animal. Te was developed during the '30 but gained popularity in herpetological studies during the early '90. To measure this index, a wealth of biophysical models had been used so far. Copper models, PVC tubes, plasticine models and data loggers are among the most used so far. However, many studies missed to calibrate the models or did not report the calibration procedure, and this experimental flaw led to several critiques. Here we present the result of a test aimed to assess if a small data logger, iButtons (Maxim/Dallas SemiconductorCorp., USA) can be used to collect information on the Te distribution for small lizards. We placed in the field 10 data loggers and 10 males of Podarcis bocagei (SVL = 59.97 ±3.96 mm). We put four lizards and four data loggers in the full shade, and the others six couples in the full sun. The test took place the 28th of April and encompassed eight hours of measurements. We regressed the Tb of the lizards against the iButton temperatures. We found a high agreement between the two data set, with a R² = 0.972. The intercept is not different from 1, the slope is between 0.94 and 0.99 (nperm = 999), and the mean difference between data loggers and Tb was 0.1 °C, with a standard deviation of 1.22. The results strongly support the use of iButton to collect data on operative environmental temperature for small lizard species.

Key words: Operative environmental temperature, iButtons, Thermal ecology, Lacertidae, Podarcis
Amphibian occupancy and pond assessment in Alentejo region (Portugal)

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Alentejo is a rural region spread in the southern third of Portugal. Here temporary ponds may be found across the landscape, though they are still poorly known or protected. In this study 43 temporary ponds were surveyed for amphibian presence and indicator plant communities. We also registered 13 features to describe each pond. Regionally 13 amphibian species may be found in the Alentejo. However, we registered between 2 and 8 species per pond (average 3.86±1.44); larger ponds tended to be more species rich. The most present species in the ponds were Pelobates cultripes (77.0%), Hyla meridionalis (69.8%) and Pleurodeles waltl (58.1%), while the less recorded were Salamandra salamandra and Alytes cisternasii (both with 2.3%). Statistical analyses were also conducted to find out which variables could explain the pond variance. The results showed that three principal components (PCA) explain 53.8% of variance based on the 13 pond features, but only 7 factor loadings (> 0.60) tend to split the ponds. PC1 splits the ponds according to the main substrate of the pond bed: positive side of the axis shows the clay (mud) substrate with the presence of carbon in the soil, against the ponds with sandy bed (negative signal). Otherwise PC2 drives the ponds to the negative side of its axis based on both pond area and plant richness (two correlated variables r = 0.54). Water chemistry also contributed to PCA discrimination, but showing a tendency to thrust aside the ponds with higher level of dissolved oxygen (PC1), slightly acid water (PC2) and lower total hardness (PC3).

Key words: amphibians, temporary ponds, conservation, Portugal
Spatial ecology of king cobras (*Ophiophagus hannah*) in Northeast Thailand

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Iconic throughout its range, the king cobra (*Ophiophagus hannah*) is the largest venomous snake in the world. Although king cobras are listed as Vulnerable under the IUCN Redlist, their life history is almost completely unknown. We assessed king cobra spatial ecology in the Sakaerat Biosphere Reserve in Northeastern Thailand using radio-telemetry and novel approaches to home range analysis to increase the understanding of king cobra spatial requirements. We implanted 6 male and 2 female King cobras with radio-transmitters and environmental data was collected at each new location. We used both fixed-kernels and Brownian bridge movement models (BBMM) to determine the home range and habitat utilization for each tracked individual. The home range delineated through BBMMs clearly shows the connections between utilized habitats better than the fixed kernel method, as well as being more consistent with field observations of habitat selection. With both methods, we found clear differences in home range size between individuals, with the snakes with smaller body sizes exhibit small home ranges. We also determined habitat preferences for each snake using land-use maps generated by the Thai government for the Sakaerat Biosphere Reserve. We determined the available and preferred habitats within the home ranges delineated using kernels and BBMMs. Our work reveals that King cobras can live in close proximity to humans, and utilize human-disturbed landscapes. As king cobras exhibit large home ranges and varied habitat utilization, using this species as a potential flagship or umbrella species will provide protection of large are tracts of habitat for numerous endemic and Red-Listed species living in both the Sakaerat Biosphere Reserve and in the surrounding areas.

**Key words:** Brownian bridge movement models, habitat utilization, home range, Sakaerat Environmental Research Station
How important is adaptation for alien species? A case study with insular *Podarcis sicula*

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Biological Invasions provide an excellent opportunity to study ecological and evolutionary processes beneath the adaptation of a species to a new environment, due to the stochastic processes and drastic habitat shifts that small introduced populations may suffer. The Italian wall lizard, *Podarcis sicula*, has been introduced in multiple areas inside and outside Europe where has established populations even under environmental conditions which may be very different from those in the native range. There is also local evidence of fast morphological adaptation in a small islet involving shifts, in few generations, affecting head morphology, bite strength and digestive tract. This makes this species a good model to understand the process of local adaptation and to test its repeatability. We present the results of an on-going project focused on four parallel island systems: Tuscany, Aeolian, Egadi and Croatian Islands (Otok Kluda, Otocic Pijavika, Pod Mrcaru, Pod Kopiste and Susac). Since such systems differ in colonisation times, it is expected to identify both the short- and long-term changes. We are using ddRAD analysis to identify similar SNPS between populations and to assess the genes showing signs of non-neutral variation. In parallel, stable isotopes analysis will allow assessing the degree of dietary shift while ecophysiological experiments will determine eventual changes in fundamental niche. This way, we expect to understand how adaptation contributes for a successful invasion when alien species meet a new environment and, hence, to develop a strong theoretical framework in invasion ecology to be applied in managements. The preliminary results on ddRAD experiments and stable isotope analysis in the pilot Tuscany system are presented.

**Key words:** *Podarcis sicula*, Mediterranean Islands, ddRAD, Stable Isotopes, Alien Species, Adaptation
Ontogenetic variation of venom composition and protease activity in Halys pit viper (Gloydius halys)

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We used a local isolated population of Halys pit viper (Gloydius halys) as a model system to study ontogenetical changes of snake venom. Snakes were captured at the single field site and delivered to a laboratory, where they were kept for several days, measured, milked, and individually marked by scale clipping. The total sample size comprised 68 individuals of different age. All snakes were released at the place of initial capture. The individual venom samples were frozen and lyophilised immediately after extraction, and stored at 4 ºC. The amount of protein in the venoms was estimated using the method of Bradford. Protease activity towards azocasein was measured by a colorimetric assay under the range of temperatures (0, 8, 16, 26, 37, 42, 52, and 62 ºC) and pH 7.5. Reverse-phase HPLC separation was used to investigate ontogenetic differences of venom composition. There were no sex differences in protease activity of G. halys venom (p = 0.676). Thus, we carried out a combined analysis of male and female samples. Linear regression analysis revealed significant effect of SVL (i.e. age) on venom protease activity in the range of temperatures (37 ºC r^2 = 0.275, p = 0.015; 42 ºC r^2 = 0.382, p = 0.003; 52 ºC r^2 = 0.515, p < 0.001; 62 ºC r^2 = 0.555, p < 0.001). Hence, immature individuals have more prominent protease activity. EDTA inhibition assays showed that most of the protease activity is due to activity of metalloproteases (mean inhibition effect is 98.6%). Analysis of HPLC profiles revealed peaks (i.e. venom components) linearly associated with SVL, while some other peaks occurred exclusively in a certain age group. Further analysis will be undertaken to identify these components. This work was supported by Russian Foundation for Basic Research (RFBR grant 14-04-32272_мол_а).

Key words: venom, protease activity, HPLC, ontogenetic variability, Gloydius halys
Heterochrony in the evolution of Podarcis lizards (Lacertidae): insights from cranial osteology

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Heterochrony is widely accepted to be an important factor in lacertid lizards evolution. It was suggested that hypermorphosis (extension of growth along the same ontogenetic trajectory) is responsible for sexual dimorphism and interspecific morphological differences in Podarcis lizards. Paedomorphosis – truncation of growth – received less attention. Podarcis erhardii and P. cretensis are morphologically very similar to each other and were until recently regarded as conspecific. However, molecular analyses suggest that these two lineages diverged in the late Miocene, over 9 million years ago. Cranial osteology indicates that these species differ in several characters, despite lack of pronounced morphological differences. All of the studied P. cretensis specimens (collected at the Elafonisos island) show tripartite occipital condyle, low ascending process of the supraoccipital and an open parietal fossa. These are all characters seen in earlier ontogenetic stages of lacertids or adult specimens of paedomorphic taxa, such as Acanthodactylus and Parvilacerta parva, but they are absent in larger specimens of P. erhardii. Morphometric analyses suggest that sexual dimorphism of P. cretensis is less developed which may also be a result of paedomorphosis. Heterochrony could also play a role in the evolution of P. hispanicus*. This is supported by the absence of median crest of the parietal and very slender posterior processes of the parietal in both sexes – characters which can be observed in some paedomorphic lacertids.

Key words: paedomorphosis, morphometrics, skull, sexual dimorphism
Potential influence of climate conditions on body condition of Syrian spadefoot toads, *Pelobates syriacus*

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In the context of the predicted changes in the global climate and its impact on amphibian populations variation in body condition provides a useful measure. Body condition is a crucial determinant of an individual’s fitness, influenced by many important ecological parameters. We used data from a field study of a Syrian spadefoot toad (*Pelobates syriacus*) population from the southern part of Danube Delta Biosphere Reserve, Romania, conducted between 2011 and 2015. A minimum of 150 individuals were measured each year, at crucial ecological moments (before and immediately after hibernation and after the breeding season), with a total sample size of 755 juveniles and 1156 adults. We investigated the relationship between body condition and the local climatic condition experienced: mean monthly temperature and precipitation during the previous active period, during hibernation, and for the month preceding breeding. The results showed significant interannual variation in body condition and in the mean temperature and precipitation for the month preceding breeding, but no corresponding effect was found. Our study did not find support for a relationship between body condition and climatic conditions experienced before and after hibernation, during the previous years or during the month preceding breeding. However, the results must be treated with caution because the short period over which we collected the data may preclude rigorous statistical testing.

**Key words:** body condition, *Pelobates syriacus*, climatic conditions, hibernation, breeding
Seasonal life history and activity pattern in a population of *Vipera berus bosniensis*

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Balkan adder population characteristics and peculiarities remain poorly understood. This study focused on a mountainous population in Vitosha Mt. (Western Bulgaria). The long-term study has been ongoing since 2010. So far, a dataset with 161 specimens (both adults and juveniles) was collected. Field research was focused on 1.7 km\(^2\). When analyzing data, to better characterize seasonal activity preferences, individual’s log transformed weight was regressed against logSVL to evaluate the body condition index (BCI). Additionally, body temperature (Tb, also log transformed) was regressed against important environmental variables such as substrate and air (at 15 cm) temperatures (logTs and logTa), collected in the field. This way, temperature condition index (TCI) was implemented. As expected, the air temperature taken at 1 meter height was with much lower impact on the Tb, therefore it was not used to calculate TCI. Monthly activity pattern changes were well expressed for all age and sex classes. Mature males were with very low summer activity, unlike mature females that were with the highest activity during that period. The proportion of encountered pregnant females was highest in August. Immature males and females activity was highest in September, when the newborns were the most prevalent. In the spring and summer their encounter rate was very low. Mature individuals post-breeding season clearly diverge in their BCI amplitude. Females who get pregnant keep their BCI higher but with greater amplitude than non-pregnant specimens, especially in August-September before they give birth. In respect to TCI mature females were with higher scores than males in early spring, but later (in June) males were with higher scores and considerably greater amplitude of TCI. Mature females amplitude progress of TCI values during gestation in summer showed similar tendencies to that of non-pregnant individuals. Both BCI and TCI seem to be useful tool for tracing the adder seasonal history.

**Key words:** adder, conditional index, seasonal changes
Molecular identification of the captive Philippine crocodiles (*Crocodylus mindorensis*) for an ex-situ conservation

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The current assessment of crocodilians recognizes them as a group under serious threat due to their habitat destruction and illegal poaching for their lucrative products. In addition to these threats, the elimination of spatial boundaries through modern anthropogenic pressures has facilitated hybridization in crocodiles by bringing together species that would otherwise not breed due to lack of opportunity. The targeted species of this study, the Philippine crocodile (*Crocodylus mindorensis*), is critically endangered and listed in CITES Appendix I. This study deals with a significant portion of the Philippine crocodile captive population in Europe based on mtDNA, nucDNA and microsatellites. The species genetical purity of all 13 specimen of *C. mindorensis* was successfully determined on the ground of testing maternally inherited mitochondrial gene cytochrome *b* and and D-loop loci from the mitochondrial control region as well as two nuclear markers, LDHA and C-mos. Also the purity of 11 out of 13 mentioned individuals on the basis of several (7) microsatellite loci was established, while confirming a supposed hybrid origin of two crocodiles with mixed morphotype. We also detected a broader range of allele sizes than were known for these loci so far. Based on the obtained genetic characters I defined a likely kinship of this group of crocodiles and subsequently suggested an optimal breeding management within the *ex-situ* conservation efforts.

**Key words:** Philippine crocodile, purity, kinship, genes, microsatellites
Reptile and Amphibian Collections at the Natural History Museum, London: A brief history with comments on modern accessibility and relevance

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The reptile and amphibian collection at the Natural History Museum in London, UK (NHM) is one of longest maintained herpetology collections in the world (from the late 18th century). The collection includes a large number of type specimens and important historical collections made by Charles Darwin, David Livingstone, Lionel Walter Rothschild, John D. Godman, Malcolm A. Smith, Frank Wall, Fernand Lataste, Jacques von Bedriaga and many others. Recently, the NHM herpetology group has initiated efforts to generate digital records for the collection. Using up to 86,675 digital records we approximated the geographic and temporal distribution of the NHM herpetology collections. We found that African and Asian taxa were strongly represented (20,000+ records each) while Oceanian, South American, North American, and European taxa all featured fewer than 7,000 records each. The earliest digitized record was from 1816 and the most recent from 2013. The vast majority of records are from specimens accessioned before 1950 (70,000+ records). This bias towards older specimens highlights the utility of the collection for research related to ancient DNA, long-term conservation and ecological monitoring, and systematics. Given these attributes, we discuss ways to engage in collaborative or independent research projects that use and enhance the collection.

Key words: Museum science, specimen-based research, archival DNA
Passive trapping manipulations for maximizing herpetofauna captures from mix deciduous forest and dry dipterocarp forest in Sakaerat Biosphere Reserve, Thailand

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Herpetofaunal communities are difficult to sample because many species have low detection probability. Passive trapping is an important method for studying herpetofaunal diversity, however there are still limitations. We assessed different trap entrance designs for using funnel traps connected to drift fences in mixed deciduous forest and dry dipterocarp forest at the Sakaerat Environmental Research Station, Nakhon Ratchasima, Thailand from December 2013 to March 2014. We constructed 5 “Y”-shaped drift fence arrays in each forest type, with 12 double funnel traps each. Every drift fence array had three traps that were treated with three types of entrance designs. The control used the conventional entrance design with traps attached along the drift fence. We created a winged entrance that included an additional 1 m of drift fencing extending out from the exterior of the trap. We used the same wing entrance but included a hood that covered the area in front of the trap between the wing and the main drift fence as the final treatment. We randomly assigned entrances types to each trap. We captured 276 reptiles and 27 amphibians in total over 4,620 trap nights. We used Kruskal-Wallis tests as the data was non-parametric. We found no significant difference for herpetofauna between manipulations, but did have significantly more herpetofauna captures between forest types. We assessed snake captures separately as a model group for difficulties and biases in passive trapping design. Snakes species richness was recorded highest from hooded traps with 11 species, compared to 9 species for the wing and control entrances. Additionally, the largest snake captured, a *Boiga siamensis* 1.5 m in total length, was from the hooded treatment. We expect hooded treatment to show greater representation of the faunal community by capturing more species and with less bias towards smaller bodied snakes, with additional sampling.

**Key words:** biodiversity, community, methods, sampling
Preliminary spatial ecology of green pit vipers (\textit{Trimeresurus macrops} and \textit{T. vogeli}) in Sakaerat Environmental Research Station, Nakhon Ratchasima, Thailand

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Little is known about the ecology of green pit vipers (\textit{Trimeresurus} sp.) in the wild. Preliminary research conducted at the Sakaerat Research Station in Northeast Thailand investigated the spatial ecology of this unique and locally medically important group of snakes through radio telemetry. Three female Big-eyed (\textit{T. macrops}) and two female Vogel’s (\textit{T. vogeli}) green pit vipers were tracked once during the day and then once at night in the same dry evergreen forest fragment from July 2014 to the end of April 2015 (approximately 30 weeks). \textit{T. vogeli} were tracked through all three seasons (rainy, cold, and hot), \textit{T. macrops} were primarily tracked during the cold season. Mean displacement and home range size varied between seasons, and \textit{T. macrops} exhibited smaller overall home ranges than \textit{T. vogeli} (approximately mean 0.5 and 4.0 ha overall, respectively). Both green pit viper species were found to have small home ranges and limited displacement patterns when compared to other viperids. Limited displacement and small home range suggest that \textit{T. macrops} and \textit{T. vogeli} could be vulnerable to fragmentation at relatively small spatial scales. Prey, bird mobbing, and conspecific interactions as well as successful predation events with tracked vipers were recorded by personnel and fixed cameras during the study which require additional study as they could significantly influence the movement patterns of green pit vipers. Further research may reveal additional factors influencing the spatial ecology and subsequent niche partitioning and habitat use between the two sympatric species of green pit viper in this study.

Key words: displacement, home range, radio telemetry, Southeast Asia, viper

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The most important areas of conservation interest for amphibians and reptiles in Europe

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We performed spatial prioritisation analysis for European amphibians and reptiles with the systematic conservation planning software Marxan, mostly based on the recent article “Updated distribution and biogeography of amphibians and reptiles of Europe” (Sillero et al. 2014). We used Europe as study area with 4063 50x50 km UTM cells as planning units. The basis of the prioritisation were the IUCN conservation status and European endemity of 208 native species. We set 100% of the occurrences as targets for Critically Endangered, 75% of Endangered, 50% of Vulnerable and 10% of European endemic species, while for all others we specified at least two occurrences. We visualised these location frequency of the planning units in a GIS. Our results revealed the most important areas for conservation interest in Europe, for example North-Western Iberia and the Adriatic triangle. Our results can serve as a proxy for future large scale conservation projects.

Key words: Key Biodiversity Area, herpetofauna, biodiversity, spatial prioritisation, conservation, Marxan
Effects of exposure to salinity on the activity of tadpoles in two syntopic species of spadefoot toads (genus Pelobates)

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The distribution of anuran species depends on reproductive success, i.e. the ability of embryos and larvae to survive, develop, and metamorphose at oviposition sites. In coastal areas, aquatic habitats can be subjected to variations in salt concentration, so salinity tolerance is key to survival. In the case of closely related syntopic species living in this environment, any difference in the tolerance to salinity that one species would exhibit over the other could constitute an adaptive advantage. We investigated if long term exposure to salinity affects the survival and behaviour of the tadpoles, taking into consideration that behavioural endpoints have been used to detect impacts of contaminants even when survival is not directly impeded. We collected egg-clutches of Pelobates fuscus and P. syriacus that live syntopically in an area near the Black Sea Coast, Romania and used an experimental design with three salinity treatments (moderate – 3 ‰, high – 6 ‰ and extreme – 9 ‰ NaCl), as well as a control. Survival was strongly affected for both the extreme (no embryos hatched) and high salinity treatments (no P. fuscus tadpole reached G25 stage, and 93.33% of P. syriacus tadpoles died before reaching G43 stage), but showed no difference between moderate and control. In order to detect more subtle effects of salinity in moderate concentration, we monitored the behaviour of the tadpoles by video-tracking up to 20 tadpoles from both moderate and control treatments, once a month starting with stage Gosner 25. Our results indicate that P. syriacus tadpoles are overall more active than P. fuscus ones, and that tadpoles of P. fuscus are more sensitive to osmotic stress, showing reduced locomotor performance at 3 ‰ concentration when compared to control, while P. syriacus is not affected. The increased fitness of P. syriacus tadpoles, compared to P. fuscus ones, can partially explain the observed higher abundance of the former species in the coastal area.

Key words: behaviour, ecology, salinity tolerances, spadefoot toads
Amphibians from an tropical dry forest: Reserva Ecologica Arenillas, Ecuador

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Arenillas Ecological Reserve (Reserva Ecologica Arenillas – REA) is located in southwestern Ecuador, close to the Peruvian border, and belongs to the Tumbesian biogeographic region. This region is one of the most important areas of endemism in the world, but at the same time, one of the most threatened by increasing agriculture and livestock activities. REA is one of the last remnants of tropical dry forest at altitudes below 100 m a.s.l. The reserve has an area of 17,983 ha and was included in the Patrimony of the State Natural Areas (Sistema Nacional de Áreas Protegidas) in 2001 and designated as an Important Bird and Biodiversity Area since 2005, but it has been protected from extractive activities for almost 60 years as a military reserve. Climate is characterized by a rainy season extending from January to May and a dry season extending from June to December, with an annual mean precipitation of 667 mm, and annual mean temperature of 25°C. Few studies on the amphibians from the reserve have been made so far. Over a period of six months, in 2014 and 2015, we investigated the distribution of amphibians in the reserve using regular visual and acoustic survey techniques as well as pitfall traps. Nine species belonging to five families were inventoried, most of them active only during the rainy season. At odds with the usual tropical amphibian community structure, REA is characterized by a low number of species with a very high number of individuals. Along with species typical for the Tumbesian region, i.e. species specially adapted to dry environments, in the reserve occur also cosmopolite species which are encountered usually in rain forests.

Key words: Arenillas Ecological Reserve, Ecuador, inventory, amphibian distribution
Distribution of newly designated slow worm species *Anguis fragilis* and *A. colchica* in Central Europe confirmed by molecular methods

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Due to the development of molecular techniques in number of species, previously considered one taxa, the diversified genetic lines on a species level have been designated. Along with the systematic changes it becomes necessary to accurately describe or reconsider ranges of newly distinguished taxa. Until 2010 two species of slow worm have been recognized: *Anguis fragilis* and *A. cephallonica*. However, new studies based on the sequence of mtDNA fragments and two nuclear genes, indicated the existence of three distinct clades of species rank within the taxon — the presence in Europe of at least four species of the genus *Anguis* is now recognized in the literature. As to the distribution of two *Anguis* species, *A. fragilis* and *A. colchica*, in central Europe it has been described well in Slovakia and Czech Republic, however north from this countries only several samples from Lithuania and Poland were described. Therefore, our aim was to confirm (based on molecular data) that two species of slow worms — *A. fragilis* and *A. colchica* — are present in Poland and to present the preliminary distribution of this two newly designated species. We amplified 1200 pb fragment of ND2 of almost 100 individuals from Poland. We confirmed the occurrence of both species in Poland. We present a distribution map of *A. fragilis* and *A. colchica*. In accordance to our preliminary data the sympatric occurrence of both species has not been confirmed, but the border between the species is not of the geographical character. We overruled our preliminary hypothesis of Vistula River being the distribution barrier. For each species we present a network of haplotypes.

**Key words:** cryptic species, genetic diversity, mtDNA, phylogeography, species range
Phylogeography and population structure of the smooth snake *Coronella austriaca* (Serpentes: Colubridae) — evidence for a reduced genetic pool and a genetic discontinuity in Central Europe

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Presented study concerns both genetic structure in Central Europe and phylogeography across the range of the smooth snake (*Coronella austriaca*) — an important species from the conservation point of view. We analysed 14 microsatellite markers and a 284 bp fragment of cytochrome b. We found a deep divergence between western and south-eastern Poland suggesting at least two different colonization routes for Poland, and probably Central Europe, originating in at least two different refugia. The west/south-eastern divide was reflected in haplotype distribution, topology of phylogenetic trees, and also in the population structuring seen in the admixture analysis of microsatellite data. The well supported designation of the Western European Clade suggests that another refugium might have existed, with Western Europe being colonised from that area. We found isolation by distance, and moderate to pronounced structuring in the examined geographical demes. Our data fit the assumption of the recently suggested sex-biased dispersal, in that we found a strong divide in the maternal line, and evidence for a small but existent gene flow based on microsatellite markers. All populations examined were very similar in respect of allelic richness, observed an expected heterozygosities and inbreeding coefficients. However, some genetic characteristics were different from expected when compared with the fine-scale study of *C. austriaca* from Great Britain. We observed heterozygosity deficit, high values for inbreeding, and low values of Garza-Williamson index suggesting reduction in population size.

**Key words:** biogeography, genetic diversity, microsatellites, mtDNA, refugium
Habitat use and activity of the European glass lizard, *Pseudopus apodus* in south-east Bulgaria

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The way in which animals use the physical and biological resources in habitats is an important aspect of their behavioral ecology. The habitat use of the European glass lizard (Scheltopusik) *Pseudopus apodus* (Pallas, 1775) is not yet studied. Therefore, we studied the activity and habitat use of glass lizards in two similar in their characteristics habitats in south-east Bulgaria during the spring-summer period of 2015. Each lizard captured was marked and measured (SVL, width, weight), aged and its sex was determined, along with body temperature immediately at capture. In parallel, essential environmental parameters were taken – temperature of surrounding air, substrate and shelters, dominant plant species, and humidity. We registered when the individuals are basking, where they are hiding etc. The results demonstrated that the lizards were most active between 10:00 a.m. and 2:00 p.m. and they preferred to bask on shrub-grass vegetation, instead of rocks. These findings are discussed in relation with proximate and ultimate factors, determining the habitat use of European glass lizards. Extended knowledge of habitat use of *P. apodus* is highly relevant to its future preservation.

**Key words:** activity, Anguidae, environmental parameters, habitat preference, *Pseudopus apodus*
Sexual dimorphism in postcranial skeleton of the sand lizard
*(Lacerta agilis* L.)*

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The sand lizard (*Lacerta agilis*) is an oviparous lacertid lizard occupying a major part of temperate Eurasia. It exhibits sexual dimorphism in coloration and body shape. We investigated the differences between sexes in size and shape of postcranial skeleton. The dimensions found to be sexually dimorphic are length of limb bones, humerus and femur width, as well as epiphyses of radius and ulna. The clear pattern of sexual dimorphism observed in the sand lizard is that males have relatively longer limbs than females. It is a common pattern among several lizard species and it may be explained by the pressure for greater locomotor abilities of males. Females have also a longer tibia at given femur length. There might be two ways to explain such dimorphism. Male thigh may be longer as it is positively correlated with femoral pores size and thus enhances their chemical signaling and may be favorably sexually selected or the longer femur increase the lever moment for muscles being of the greatest importance for gait and speed. The differences in the relative epiphyses width may have not any functional nor adaptive explanation but may be secondary effect of longer period of intensive growth of female sand lizards.

**Key words:** Lacertidae, sexual dimorphism, morphometrics, osteology
Toward the population history of *Ablepharus kitaibelii* in the central part of its distribution range

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The snake-eyed skink (*Ablepharus kitaibelii* Bibron & Bory de Saint-Vincent, 1833) is the smallest scincid species in Europe. Population biology and ecology of this species remain poorly investigated. A population was studied in the central part of its distribution range (Pastrina Hill, Northwestern Bulgaria). Data presented cover two field seasons. The study site covers approximately 0.5 ha. A total of 220 specimens were captured and individually marked using a portative soldering iron. Capture-mark-recapture was performed in 6 sessions, continuing for 5–6 days each (in May, July and September). Each captured specimen was measured (SVL and tail length) and weighted. Relative age accessed by skeletochronology (stored material coming from different localities) was used as a referent parameter. The resultant dataset was analyzed with the Cormack-Jolly-Seber (CJS) model in program MARK. Body condition index (BCI) in juveniles increased proportionally to size, while in adults BCI decreased. In juveniles as opposed to adults SVL increased faster than the weight. Differentiation between the size of males and females appeared after the first hibernation. No difference in maximal attained age was found between sexes. The largest specimens from both sexes had undergone 3 hibernations, meaning they were 4 years old. Substantial differences between two years juveniles’ influx was observed. The encountered rate was low overall (0.3±0.1). CJS’s most parsimonious model, i.e. survival rate and capture probability as function of time, revealed that still more sessions are needed. It could be concluded that the studied population is characterized by substantial emigration/immigration and/or mortality rates, factors that need to be further investigated.

**Key words:** condition index, population rate, recapture, skeletochronology
Niche differentiation and feeding ecology of two sympatric species of *Xenopus* (Anura: Pipidae) from the Western Cape Province of South Africa

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The endangered, endemic Cape Platanna (*Xenopus gilli*) and the African clawed frog (*Xenopus laevis*) sympatrically inhabit a variety of freshwater habitats across the southwestern Cape, South Africa. In order to quantify the invasive species’ impact on sympatric populations of *X. gilli*, an assessment of the dietary requirements, patterns of interspecific competition and niche differentiation was conducted at two study sites, comprising three permanent and five temporary water bodies. A total of 399 stomach contents of *X. laevis* (183) and *X. gilli* (216) were obtained using a stomach flushing technique. Food preferences were determined using the Electivity index (E*) and the index of relative importance (IRI), while niche breadth for *X. gilli* and *X. laevis* was assessed using both, the Shannon as well as the Simpson index. Niche overlap between both species was determined using the Pianka index. Both species were found to utilize similar nutrient resources confirming interspecific competition. While both species were found to consume large amounts of tadpoles belonging to different amphibian species, *X. laevis* also feeds on adult *X. gilli* proving *X. laevis* to represent a direct threat for *X. gilli*. Trophic niche breadth was found to be larger in *X. gilli* than in *X. laevis* suggesting *X. gilli* to maintain a more generalistic feeding pattern. Niche overlap was 50% for the first study site; however in the second site niche overlap was 100% suggesting both species to utilize the same trophic niche.

**Key words:** Foraging generalist, niche segregation, Prey electivity, stomach flushing, trophic niche
Tadpoles of *Buergeria japonica* (Rhacophoridae) living in geothermal hot springs and streams exhibit different life history traits under different thermal regimes

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Thermal environment experienced during ontogeny have immediate effects and potentially long lasting consequences on life history traits of animals living in different environments. The rhacophorid tree frog, *Buergeria japonica*, usually breeds in cold water in ditches or small streams, but it also breeds in an unusual habitat, geothermal hot springs. One of the most distinct differences between the two breeding sites of *B. japonica* is the thermal environment where tadpoles live. We examine how different thermal regimes affected survival, metamorphic traits, and post-metamorphic morphology of *B. japonica* tadpoles. Tadpoles collected from a cold water environment and a hot spring were initially assigned to either low (25°C) or high temperature (35°C), and then some were switched between temperature levels after either 1 week (early switch) or 2 weeks (late switch). Results showed that switching temperature did not influence survival of tadpoles from a cold water environment but influenced those from a hot spring, which showed that tadpoles from a hot spring experiencing a longer cold period had poor survival. Tadpoles from a cold water environment under different thermal regimes exhibited different metamorphic traits, which showed that tadpoles exposed to a longer cold period metamorphosed later and at a bigger size with a longer body length, head width, and hind limb. However, tadpoles from a hot spring under different thermal regimes metamorphosed at different times but at a similar size with a similar post-metamorphic morphology. Our results thus show that the interpopulation difference in survival and metamorphic traits of *B. japonica* tadpoles under different thermal regimes, suggesting that the difference is probably related to adaptation to different thermal regimes of habitats.

**Key words:** hot spring, stream, thermal acclimation, survival, metamorphosis, fitness
Amphibians life strategies: Does age and parasites influence migration and spatial distribution? Example for moor frog (*Rana arvalis*)

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The hypothesis that age and parasites have impact on frogs’ migration and habitat preferences were tested using passive integrated transponder (PIT) surveys and pitfall traps for seasonal distribution. 2000 frogs were individually PIT-tagged in 4 breeding sites and frogs’ abundance was monitored in surrounding microhabitats during 3 subsequent years from early spring till late autumn. Skeletochronology was made to determine the age structure of population, relationship of age and habitat use, host age and parasites infections. Age structure shows that 60% of population consists of three old specimens, 27% are younger ones and only 13% are older than three. Age analysis in the breeding sites indicates that younger frogs migrate earlier and older specimens tend to appear later in the breeding pond. We observed animals that were more “active” (leaving the pond faster after mating and moving farther) and animals more “sedentary” that during the same time (June-July) stayed in or in a close surroundings of breeding pond. In a group of “active” frogs there are more younger specimens and in a “sedentary” group there are more older specimens than it is predicted from the age structure. Results show that forest was the most occupied postbreeding habitat with the highest animal activity in May; the least suitable seems to be natural, wet and dry grasslands. Younger specimens had higher and longer activity during the season. 180 frogs were subject to parasitological studies. *Rana arvalis*’ parasites communities were dominated by nematode species and total infection intensity increased with age. Our observation shows that realized life strategies depend on age and intensity of parasites infection.

**Key words:** *Rana arvalis*, habitat use, parasite infection, skeletochronology, life strategies
Evaluating the potential for competition between *Podarcis muralis* and *Iberolacerta horvathi* using morphology

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Competitive outcomes between lacertids also depend on the size of individuals; bigger lizards usually out-compete smaller ones in combat-like interactions. The common wall lizard (*Podarcis muralis*) and Horvath’s rock lizard (*Iberolacerta horvathi*) co-exist across the distributional range of *I. horvathi*, where syntopic populations have been found at middle altitudes. Previous studies in Northern Dinaric Mountains revealed that their fundamental niches considerably overlap, they are very similar in size and shape, and agonistic social interactions between males of both species have experimentally been demonstrated to have repercussions on thermoregulation. In this study, we used five morphological characters that represent size and ecologically relevant body shape to evaluate the potential for competition between *P. muralis* and *I. horvathi* across their area of overlap, under the hypothesis that the potential for interspecific competition would be expected to be higher if interacting species are more similar. We examined a total of 597 individuals from 40 localities for *I. horvathi* and 41 localities for *P. muralis* to first describe spatial patterns of morphology using Inverse Distance Weighted interpolation for each species separately. We then used values of the interpolated raster surfaces in a correlation analysis to compare the species’ morphology across the geographic area, while including abiotic factors (altitude and climatic variables) to account for possible environmental effects on morphological variation. Preliminary results showed that body size, head length and pileus length exhibit a latitudinal gradient in *I. horvathi*, but not in *P. muralis*. These interspecific differences in spatial patterns of morphology suggest that the potential for competition is unevenly distributed across the area, which may translate into different local competitive outcomes.

**Key words:** morphology, interspecific competition, *Podarcis muralis*, *Iberolacerta horvathi*
Selected morphometrical and ecological traits of smooth snake (*Coronella austriaca*) populations in Cracow – preliminary results

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Smooth snake (*Coronella austriaca*) is one of the rarest reptile species in Poland. Although single records of this species was reported from the city of Cracow at beginning of twentieth century, the numerous populations were not described until 2007 – 2015 period. These populations inhabit two similar, meadow and post-quarry habitats, yet differentiated in terms of the level of urbanization of surroundings. Site Tyniec is placed within and around suburban settlement surrounded by mixture of countryside and forest, while site Zakrzówek is surrounded mostly by newly urbanized areas of Cracow. Within these two sites, smooth snakes inhabits habitats with well-developed structure of open and semi-open habitats, as well as rocky slopes and bottoms of quarries. Regarding morphometrical data, mean snout-vent length of smooth snake in Zakrzówek is 59.3 cm and mean tail length is 10.4 cm. Mean body mass is 75.3 g. Head length measured to the end of parietal scale is 16.7 mm and width measured above eyes on supraocular scales is 8.4 mm. Most important threats to the smooth snake populations in Cracow are urban sprawl on adjacent suburban areas and plant succession, mainly by invasive species (e.g. *Solidago canadensis*)

**Key words:** smooth snake, Cracow, morphometry, urban
Distribution of reptile populations within urban areas of Cracow

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Cracow is one of the largest cities in Poland, and at the same time still maintains a relatively high level of biodiversity. However, in the second half of the twentieth century, the city experienced fast urban sprawl which affected local populations of wildlife, including reptiles, mainly through habitat destruction or fragmentation. Despite these negative overall trends, some habitat patches still remain and support the existence of small and probably isolated populations. Historically six species of reptiles were reported from Cracow, including five species from contemporary urbanized areas, and one, the pond turtle (*Emys orbicularis*) only from the nearby countryside. From these five species, the presence of slow worm (*Anguis fragilis/colchica*) has not been confirmed recently. Today populations of sand lizard (*Lacerta agilis*), viviparous lizard (*Zootoca vivipara*), grass snake (*Natrix natrix*) and smooth snake (*Coronella austriaca*) are reported within the urban areas of Cracow. The most common species is sand lizard (occurrence on at least seven sites), followed by grass snake (at least six sites), viviparous lizard (three sites), and smooth snake (one site). Reptile habitats in Cracow include abandoned quarries, surroundings of remnant ponds, meadow patches, oxbows and railway embankments.

**Key words:** reptiles, diversity, Cracow, urbanization, distribution
The variability of NOR patterns in the European water frogs *Pelophylax ridibundus*, *P. lessonae* and their natural hybrid *P. esculentus*

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The NOR-distribution in water frogs from Central Europe: *Pelophylax ridibundus* RR (2n=26), *P. lessonae* LL (2n=26) and their hybrid *P. esculentus* was examined. Among hybrid individuals, both diploids RL (2n=26) and triploids RRL and LLR (3n=39) can be distinguished. Diploid karyotype consists of 26 chromosomes (5 pairs of large and 8 pairs of small chromosomes) that in each taxon are very similar in shape and size. Analysis of the nucleolus organizer regions (NORs) using silver, CMA₃ and DPI stainings, and fluorescence *in situ* hybridization with 28S rDNA probe techniques showed the intraspecific polymorphism of NORs in haploid sets of chromosomes. The secondary constrictions with NORs were observed in the majority of individuals (83.5%) on the long arms of the chromosomes no. 10 in both sexes. 2.2% individuals had NOR on the short arm of the chromosome no. 2, and one individual (0.25% within all individuals) had NOR on the long arm of the chromosome no. 9. The lack of NORs per haploid set was found in 13.8% individuals. Altogether, 16.2% individuals displayed polymorphism in the localization and number of NORs. The results provided not only new data on distribution and polymorphism, but also helped understanding a specific way of genome transmission by hybrids (*P. esculentus*) during hybridogenetic gametogenesis.

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**Key words**: hybridogenesis, NOR chromosomes, karyotyping
Do temporally separated spawning seasons lead to genetic population substructure in yellow-bellied toads (*Bombina variegata*) in Salzburg, Austria

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Yellow-bellied toads (*Bombina variegata*) in Austria show a reproductive season from late April to late August. Some observations indicate, that during this period different individuals are present at a certain reproduction site, presumably forming successive genetically differentiated cohorts (GOLLMANN & GOLLMANN 2012), as it was documented for *Bufo calamita* by SINSCH (1997). This project attempted to approve the hypothesis if specific spawning behavior of *Bombina variegata* can result in a cryptic differentiation of populations and if temporally separated spawning seasons (spring/late summer) lead to genetically differentiated subpopulations. We tested two populations, Fürstenbrunn and Bürmoos, both located in the north of the federal state of Salzburg (Austria). All present adult toads were captured twice a month at the two sites. Tissue was sampled by swabbing of the buccal mucosa with sterile cotton swabs. DNA was extracted and samples were genotyped by performing amplified fragment length polymorphisms (AFLP) in order to investigate the genetic variation within and among the populations. The different hierarchical analysis of molecular variance (AMOVA) indicated that there is no significant genetical variance (0 percentage of variation, p=1) among the capture date groups (May to August) of the population at the spawning water. Furthermore non-significant pairwise differences (F\(_{ST}\)) between all individuals and a permutation test of the distance matrices “Genetic distance” and “Presence distance” did not evince correlations between genetic diversity and the capture dates. In contrast, the analysis of molecular variation among the spatial separated populations of Fürstenbrunn and Bürmoos (11 percentage of variations, p=0) and the positive pairwise differences between the individuals of each populations showed a significant genetic diversity.

**Key words:** AFLP, DNA, fingerprinting, *Bombina variegata*, population substructure
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