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Damaged by time and formalin: Using ancient DNA technology to recover century-old snake mitogenomes

Allentoft, Morten; Redsted Rasmussen, Arne; Kristensen, Hans Viborg

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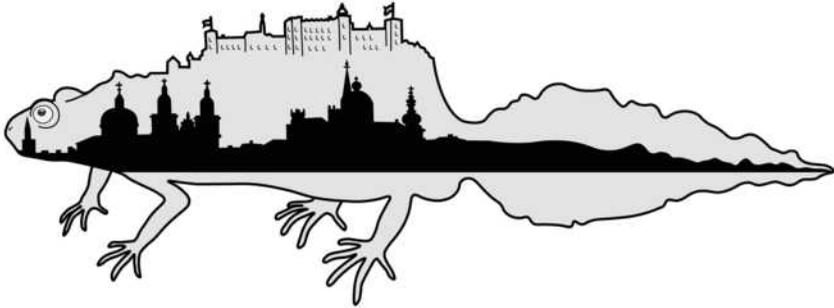
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SEH 2017

19th European Congress of Herpetology



PROGRAMME & ABSTRACTS



University of Salzburg
18th - 23rd September 2017

Chief Patron:

Astrid Rössler, Vice Governor of the State of Salzburg

Organising Institutions:

University of Salzburg, Department of Ecology and Evolution
ÖGH Austrian Society of Herpetology
Haus der Natur Salzburg, Museum of Nature and Technics
Herpetological Work Group - Haus der Natur

Local Organising Committee:

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Dominik Ankel, Tina Hametner-Rieder, Dorothee Hoffmann, Bernadette Kaufmann,
Werner Krupitz, Alexander Niedrist, Cvetka Lipovnik, Maria Müller,
Rainer Mysliwietz, Ursa Pretzl, Valerie Saliger, Mario Schweiger, Marcus Weber

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Congress Logo:

Valerie Saliger

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WELCOME

Welcome to Salzburg!

The 19th SEH European Congress of Herpetology takes place at the Faculty of Natural Sciences of the University of Salzburg. It is hosted by the University of Salzburg and Co-organised by the Herpetological Work Group of the Haus der Natur Museum of Nature and Technics Salzburg and the Austrian Society of Herpetology (ÖGH).

More than 200 participants from all continents and 37 countries have registered and contribute to this particularly diverse and significant meeting in many fields of the science of herpetology.

The scientific programme includes four invited talks with topics which will encompass many scientific fields in herpetology, as well as many geographical areas and herp groups. 129 oral presentations are scheduled in 12 regular sessions, one symposium on *Batrachochytrium salamandrivorans* and a workshop on Micro-computed tomography (Micro-CT). Also 88 posters will be presented during the two poster sessions and will be accessible throughout the congress. An open meeting of the Regional IUCN Viper Specialist Taxonomy Working Group for Europe and North Asia completes the manifold programme. There will also be a contest for the best student talk and poster with valuable prizes.

At the welcome party, the visit to the Augustiner Beer hall and at the gala dinner, there will be additional opportunities to meet each other and have inspiring talks.

As it is autumn already, we chose localities for the field excursions, where amphibian and reptile sightings are still very likely and the habitats are both picturesque and of conservation interest, also in terms of landscape, botany or ornithology. We also chose places, which are not far from the congress venue, to limit the transport time in favour of herping.

On behalf of the Local Organising Committee I wish you an inspiring and stimulating meeting.

Kind regards,
Andreas Maletzky

GENERAL INFORMATION

Venue

University of Salzburg, Faculty of Natural Sciences
Hellbrunnerstraße 34, A-5020 Salzburg

Opening Hours and Registration

| | |
|---------------------------------------|---------------|
| Monday, September 18 th | 15:00 – 21:00 |
| Tuesday, September 19 th | 08:00 – 18:00 |
| Wednesday, September 20 th | 08:00 – 19:00 |
| Thursday, September 21 st | 08:00 – 18:00 |
| Friday, September 22 nd | 08:00 – 18:00 |

Internet Access

Free Wi-Fi is available in the building

Message Board

can be found next to the registration desk

Coffee

Coffee, tea, soft drinks, mineral water, cakes and cookies will be served during the coffee breaks right in the area in front of the lecture halls.

Lunch

A few steps away from the lecture halls, also at ground floor, you can purchase reasonably priced lunch at the University Mensa. Within five minutes walking distance, there are several other opportunities to get lunch or dinner. A map with the location of these places will be part of the congress bag.

Opening Ceremony and Welcome Party

Date: Monday, September 18th

Time: 18:00 – 21:00

Place: University of Salzburg, Faculty of Natural Sciences, Hellbrunnerstraße 34, A-5020 Salzburg, ground floor, Lecture Hall 402 (blue) and Aula

Admission is free for all registered participants and accompanying persons.

Beer hall

Date: Wednesday, September 20th

On Wednesday participants can join us for a beer or two in Austria's biggest beer tavern, the famous Augustiner Brewery Mülln with tradition back to the year 1621. The **beer** is drawn **from wooden barrels** and served in stone jugs, or steins. Regional and traditional dishes can be bought directly from various food stands in the "Schmankerlgang" (Delicatessen arcade) which is set up to resemble a traditional marketplace.

Please register at the congress office and get detailed information.

Congress Photo

A picture of all participants will be taken on Wednesday, September 20th.

Time: 12:30

Meeting Place: University Aula, in front of the two lecture halls 402 (blue) and 403 (green)

Gala Dinner:

Date: Thursday, September 21st

Time: 19:30 – 22:30

Place: Restaurant Stieglkeller Salzburg, Festungsgasse 10, 5020 Salzburg – on the foot of the fortress in the heart of the historic old town.

Meeting Places: 19:00 Aula of the University or 19:20 Kapitelplatz, historic old town

Admission only with ticket

FIELD EXCURSIONS

Excursion 1 – Rivers and Bogs

Date: Saturday, September 23rd, *Departure Time:* 08:30 - *Arrival Time:* 17:00

Meeting Place: In front of the main university entrance

Admission only with ticket – there is a limited number of tickets available still, price: 20 EUR
We will visit Protected Areas in the lowlands north of the city of Salzburg (Weidmoos, Ibmer Moor and Ettenau) in the states of Salzburg and adjacent Upper Austria. Lunch and Bus Transport is provided. The tour will be guided by Andreas Maletzky.

Excursion 2 – Calcareous Alps

Date: Saturday, September 23rd, *Departure Time:* 08:30 - *Arrival Time:* 17:00

Meeting Place: In front of the main university entrance

Admission only with ticket – there is a limited number of tickets available still, price: 20 EUR
We will visit important herp areas in the Calcareous Alps about 30 minutes south of the city of Salzburg. Lunch and Bus Transport is provided. The tour will be guided by Werner Krupitz.

City night herp excursion

Date: Tuesday, September 19th, *Time:* 20:00

We will visit herp sites in the town center. As the exact programme is weather dependent, please ask and register at the congress office.

SPECIAL INVITATIONS

Free entrance to the Haus der Natur Museum of Nature and Technics

Thanks to our partner, the museum, all congress participants and accompanying persons can visit the museum exhibitions (including Aquarium, Reptile Zoo and Science Center) for free.

Adress: Museumsplatz 5, 5020 Salzburg (in the historic old town)

Admission: by showing your congress badge

ACCOMPANYING PERSONS

Accompanying persons are kindly invited to join the Opening ceremony and Welcome Party and also have free entrance to the Museum Haus der Natur.

PRESENTATION GUIDELINES

Posters

Poster size: DIN A0 (841 x 1189 mm)

Set up and removal: Poster can be set up starting from Monday, September 18th, 15:00 and must be removed until Friday, September 22nd 16:00.

Poster sessions:

Session 1: Tuesday, September 19th, 17:15-18:00

Session 2: Thursday, September 21st, 17:15-18:00

Venue: Aula of the University

Posters will be on display throughout the congress. Authors are responsible for setting up and removing of posters. Materials for setting up are provided at the congress office.

Oral presentations

Length: 15 minutes plus 5 minutes discussion = 20 minutes in total

File Format: Presentations can be provided as .ppt, .pptx or .pdf-files. Please contact the technical support for additional requests

We request to upload your presentations in time, ideally before the beginning of the morning or afternoon sessions, in which the presentation is scheduled.

AWARDS

There will be awards for the best poster and oral presentation of students with valuable prizes.

VENDORS

The Chimaira Buchhandels GmbH will be present throughout the whole congress and offer a combination of new publications and antiquariate stock.

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PROGRAMME

| PROGRAMME OVERVIEW | | | | | | | | | |
|-------------------------------|--|----------------------------------|--|-------------------------------|--|--|---|--|--|
| Monday, Sep. 18 th | Tuesday, Sep. 19 th | Wednesday, Sep. 20 th | Thursday, Sep. 21 st | Friday, Sep. 22 nd | Saturday, Sep. 23 rd | | | | |
| 08:00 | Registration Desk | | | | | | | | |
| 08:30 | Plenary 1: N. Poytkov 2a: Anuran Larvae 1 1a: Population Genetics, Genetic Diversity 1 | | Plenary 2: E. Ringler 4b: Phylogeography of European Reptiles & Amphibians 2 5: Neocoa & Invasive Species | | Plenary 3: M. Denoel 9a: Diseases & Anomalies 1 6c: Ecology and Behaviour of Reptiles 3 | | Plenary 4: M. Schindler 12a: Practical Conservation 1 13a: Evolution & Development 1 | | |
| 09:20 | | | | | | | | | |
| 10:40 | Coffee Break | | | | | | | | |
| 11:10 | 1b: Population Genetics, Genetic Diversity 2 2b: Anuran Larvae 2 4c: Phylogeography of European Reptiles & Amphibians 3 | | 6a: Ecology & Behaviour of Reptiles 1 8b: Ecology & Behaviour of Amphibians 2 | | 9b: Diseases & Anomalies 2 Micro CT Workshop | | 12b: Practical Conservation 2 13b: Evolution & Development 2 | | |
| 12:30 | Lunch break | | | | | | | | |
| 13:45 | 3a: Morphology, Anatomy & Physiology 1 Viper Specialist Group Workshop 1 | | 7a: Phylogeny, Taxonomy & Systematics 1 6b: Ecology & Behaviour of Reptiles 2 | | 10a: Bsal 1 – Symposion 8c: Ecology & Behaviour of Amphibians 3 | | 12c: Practical Conservation 3 13c: Evolution & Development 3 | | |
| 15:05 | Coffee Break | | | | | | | | |
| 15:35 | 4a: Phylogeography of European Reptiles & Amphibians 1 3b: Morphology, Anatomy & Physiology 2 7b: Phylogeny, Taxonomy & Systematics 2 Viper Specialist Group Workshop 2 | | 8a: Ecology & Behaviour of Amphibians 1 11: Biogeography, Diversity & Distribution | | 10b: Bsal – Symposion 2 11: Biogeography, Diversity & Distribution | | 16:00 Council Meeting 2 15:30 OGH Field Herpetologists Meeting | | |
| 17:15 | 17:15 Poster Session 1 17:15 SEH Ordinary General Meeting 18:00 SEH Council Meeting 1 20:00 City Herp Night Excursion | | | | | | | | |
| 18:00 Opening & Welcome Party | 17:15 Poster Session 2 17:15 Closing Ceremony | | | | | | | | |
| | 19:30 Beer Tavern 19:30 Gala Dinner | | | | | | | | |

Tuesday, September 19th – Morning

| | | |
|----------------------|--|--|
| 08:30 – 09:15 | Plenary Lecture: Nikolay Poyarkov. Herpetofaunal diversity and endemism in Indochina: new discoveries and biogeographic patterns Chair: Peter Kaufmann | |
| | Room 1 | Room 2 |
| 09:20 – 10:40 | Session 1a: Population Genetics, Genetic Diversity Chair: Peter Mikuliček | Session 2a: Anuran Larvae Chair: Arne Schulze |
| 09:20 – 09:40 | Peter Trontelj, Valerija Zakšek: A leap forward in the conservation genetics of <i>Proteus</i> populations | Arne Schulze, Jörn Köhler, Stefan Lötters, Bruno Viertel & Michael Veith: Hidden treasures: old museum tadpole collections in the light of modern scientific research |
| 09:40 – 10:00 | Valerija Zakšek, Grega Makovec, Peter Trontelj: Secondary contact and limited hybridization of two distinct <i>Proteus anguinus</i> lineages in the underground of the Classical Karst | Jan M. Kaczmarek, Mikofaj Kaczmarek, Janusz Kloskowski: A matter of proportion? Associational effects in tadpole-fish predator system |
| 10:00 – 10:20 | Alejandro Ibáñez, Molly Bletz, Miguel Vences, Sebastian Steinfart: Gut microbial diversity is related to host genetic diversity but is not affected by starvation during an el Niño event in Galápagos marine iguanas | Veronika Bókony, Zsannett Mikó, Ágnes M. Móricz, Dániel Krüzelyi, Attila Hettyey: Chronic exposure to a glyphosate-based herbicide makes common toad (<i>Bufo bufo</i>) larvae more toxic |
| 10:20 – 10:40 | Hisanori Okamiya, Hirotaka Sugawara, Tamotsu Kusano: Loss of genetic diversity prevents adaptation at the margin of distribution in a lentic salamander | Dóra Holly, Zsannett Mikó, Attila Hettyey: Effects of a glyphosate-based herbicide on agile frog (<i>Rana dalmatina</i>) tadpoles exposed to free-ranging predators |
| 10:40 – 11:10 | Coffee Break | |
| 11:10 – 12:30 | Session 1b: Population Genetics, Genetic Diversity Chair: Peter Mikuliček | Session 2b: Anuran Larvae Chair: Arne Schulze |
| 11:10 – 11:30 | Lysenko M., Zinenko O., Joger U.: Genetic diversity of <i>Vipera berus nikolskii</i> and <i>Vipera renardi</i> in Eastern Ukraine and Southern Russia | Hettyey A., Úveges B., Bókony V., Szederkényi M., Mahr K, Móricz ÁM, Drahos L, Capon RJ, Hoi H, Van Buskirk J: Predator-induced changes in chemical defences of larval common toads (<i>Bufo bufo</i>) |
| 11:30 – 11:50 | Zinenko O., Strugariu A., Joger U.: New mitochondrial haplotype from <i>Vipera berus</i> group in Southern Romania | Bálint Úveges, Veronika Bókony, Ágnes M. Móricz, Attila Hettyey: Competition induces increased toxin synthesis in tadpoles of the common toad (<i>Bufo bufo</i>) |
| 11:50 – 12:10 | Dedukh D, Chmielewska M, Rozenblut-Kościsty B, Kolenda K, Kazmierczak M, Rymin S, Ogielska M, A. Krasikova: Micronuclei include genome eliminated during gametogenesis of di- and triploid interspecies hybrids from <i>Pelophylax esculentus</i> complex | Katharina Ruthsatz, Nikita M. Sabatino, Laura I. Becker, Janica Reese, Lisa Hartmann, Kathrin H. Dausmann, Myron A. Peck, Julian Glos: Environmental stress as an endocrine disrupter in tadpoles of <i>Xenopus laevis</i> and <i>Rana temporaria</i> |
| 12:10 – 12:30 | Gregorio Sánchez-Montes, Jinliang Wang, Arturo H. Ariño, José L. Vizmanos, Íñigo Martínez-Solano: Applying integrative demography to obtain reliable effective/census size ratios in pond-breeding amphibians | |
| 12:30 – 13:45 | Lunch Break | |

Tuesday, September 19th – Afternoon

| | Room 1 | Room 2 | Room 3 |
|----------------------|--|---|---|
| 13:45 – 15:05 | Session 1c: Population Genetics, Genetic Diversity Chair: Judit Vörös | Session 3a: Morphology, Anatomy & Physiology Chair: Wolfgang Böhme | Viper Specialist Group Workshop |
| 13:45 – 14:05 | Michal Benovics, Daniel Jablonski, Peter Mikuliček: Genetic diversity of <i>Pelophylax</i> species in south-western Balkans | Natalia B. Ananjeva & Dmitry A. Gordeev: To the problem of caudal autotomy and regeneration in Asian mountain agamas | Chair: Jelka Crnobrnja-Isailovic |
| 14:05 – 14:25 | Hossein Javanbakht, Flora Ihlow, Daniel Jablonski, Pavel Široký, Uwe Fritz, Dennis Rödder, Mozafar Sharifi, Peter Mikuliček: Genetic diversity and Quaternary range dynamics in Iranian and Transcaucasian tortoises <i>Testudo graeca</i> | Samantha McCann, Georgia Kosmala, Matthew Greenlees & Richard Shine: Physiological plasticity in a successful invader: rapid acclimation to cold occurs only in cool-climate populations of cane toads (<i>Rhinella marina</i>) | |
| 14:25 – 14:45 | Jonathan Remon, Michel Baguette, Sylvain Moulherat: Genetic structure of the grass snake (<i>Natrix natrix</i>) and the midwife toad (<i>Alytes obstetricans</i>) in a landscape fragmented by five large-scale transportation infrastructures | Stanislaw Bury, Mariusz Cichoń, Ulf Bauchinger, Edyta T. Sadowska: The effect of thermal acclimation on metabolic rate, damage load and antioxidant capacity in the grass snake (<i>Natrix natrix</i>) | |
| 14:45 – 15:05 | Cäcilia Spitzweg, Margaretha D. Hofmeyer, Uwe Fritz, Melita Vamberger: Phylogeography and population genetics of the angulate tortoise <i>Chersina angulata</i> | Tijana Vučić, Maša Sibinović, Tanja Vukov, Nataša Tomašević-Kolarov, Milena Cvijanović, Ana Ivanović: Ontogeny of head shape in <i>Triturus</i> newts: geometric morphometric approach | |
| 15:05 – 15:35 | Coffee Break | | |
| 15:35 – 17:15 | Session 4a: Phylogeography of European Reptiles and Amphibians Chair: Melita Vamberger | Session 3b: Morphology, Anatomy & Physiology Chair: Wolfgang Böhme | Viper Specialist Group Workshop |
| 15:35 – 15:55 | Judit Vörös: Importance of the Carpathian Basin in phylogeography of amphibians | Rok Kostanjšek, Ylenia Prodan, Peter Trontelj: Comparative analysis of skin bacteriome from four natural populations of <i>Proteus anguinus</i> in Slovenia | Chair: Jelka Crnobrnja-Isailovic |
| 15:55 – 16:15 | Larissa Kupriyanova, Tom Kirschey & Wolfgang Böhme: Biogeography of the viviparous lizard, <i>Zootoca vivipara</i> Lichtenstein, 1823 (Squamata: Lacertidae) in central Europe and possible re-colonization of the Baltic Sea basin and the Balkans: new karyological evidence | Marco Mangiacotti, Alan Coladonato, Mattia Falaschi, Stefano Pezzi, Lorenzo Balestrazzi, Marco Fumagalli, Marco A.L. Zuffi, Stefano Scali, Xavier Bonnet, Roberto Sacchi: Seasonal T-level fluctuations and protein content of femoral gland secretions in the common wall lizard (<i>Podarcis muralis</i>) | |
| 16:15 – 16:35 | Lucio Bonato, Andrea Corbetta, Giovanni Giovine, Enrico Romanazzi, Emina Šunje, Cristiano Vernesi, Barbara Crestanello: Isolated populations of <i>Salamandra atra</i> at the southern periphery of the species range: genetic variation and evolutionary differentiation | Elif Yıldırım, Eyup Başkafe, Yusuf Kumlucaş, Çetin Ilgaz, Kamil Candan, Elnaz Najafi Majd, Uğur Kaya: Osteological and geometric morphometric comparison of five species of <i>Rana</i> and <i>Pelophylax</i> (Anura: Ranidae) | |
| 16:35 – 16:55 | Sarah Kieren, Michael Veith: The evolutionary history of Mediterranean amphibians - a comparison of different calibration approaches | Grigoris Kapsalas, Kallirroi Papadaki, Efstratios D. Valakos, Panayiotis Pafilis: Drivers of evaporative water loss in <i>Podarcis</i> lizards from Greece | |
| 16:55 – 17:15 | Miguel Vences, Ariel Rodríguez, James D. Burgon, Mariana Lyrá, Iker Irisarri, Denis Baurain, Leon Blaustein, Bayram Göcmen, Sven Künzel, Barbara Mable, Arne W. Nolte, Michael Veith, Sebastian Steinfartz, Kathryn R. Elmer, Hervé Philippe: Inferring the shallow phylogeny of true salamanders (<i>Salamandra</i>) by multiple phylogenomic approaches | Evgeny S. Roitberg, Valentina F. Orlova, Valentina N. Kuranova, Nina A. Bulakhova, Galina V. Eplanova, Oleksandr I. Zinenko, Oscar Arribas, Sylvia Hofmann, Katarina Ljubisavljević, Vladimir P. Starikov, Lukáš Kratochvíl, Henk Strijbosch, Olga A. Leontyeva & Wolfgang Böhme: Geographic Variation in adult body length and sexual size dimorphism in the lizard, <i>Zootoca vivipara</i> : testing the effects of reproductive mode, lineage, and climate | |
| 17:15 – 18:00 | Poster Session 1 | | |
| 18:00 – 20:00 | SEH Council Meeting 1 | | |

Wednesday, September 20th – Morning

| | | |
|----------------------|--|--|
| 08:30 – 09:15 | Plenary Lecture: Eva Ringler: Why to not study poison in poison frogs Chair: Nikolay Poyarkov | |
| | Room 1 | Room 2 |
| 09:20 – 10:40 | Session4b: Phylogeography of European Reptiles & Amphibians Chair: Uwe Fritz | Session 5: Neozoa & Invasive Species Chair: Peter Kaufmann |
| 09:20 – 09:40 | Fernando Martínez-Freiria, Inês Freitas, Marco A. L. Zuffi, Philippe Golay, Sylvain Ursenbacher, Guillermo Velo-Antón: Comparative evolutionary history of the Western Mediterranean vipers, <i>Vipera aspis</i> and <i>V. latastei-monticola</i> | Samuel Pinya, Víctor Colomar, Gabriela Picó, Nil Lassnig, Jorge Enrique Moreno, Joan Mayol: Population traits and diet of the introduced population of the ladder snake (<i>Rhinechis scalaris</i>) at Formentera (Balearic Islands, Spain) |
| 09:40 – 10:00 | Marco Dinis, Khaled Merabet, Fernando Martínez-Freiria, Sebastian Steinfartz, Miguel Vences and Guillermo Velo-Antón: Allopatric divergence in a North African Palearctic relict: the role of environmental factors and niche conservatism in shaping the genetic structure of <i>Salamandra atra</i> | Gregory S. Clarke, Benjamin L. Phillips & Richard Shine: The effects of invasion history on the intraspecific competitive ability of cane toads (<i>Rhinella marina</i>) in Australia? |
| 10:00 – 10:20 | Sylvain Ursenbacher, Bettina Erne, Maxime Chèvre: Different (sub)species or not different (sub)species? - study of the contact zone between two taxa of <i>Vipera berus</i> and <i>Natrix natrix</i> in Switzerland | Aija Pupina, Mihails Pupins: Adult <i>Bombina orientalis</i> predate young whitebaits of invasive fish <i>Perccottus glenii</i> |
| 10:20 – 10:40 | Guillermo Velo-Antón, André Lourenço, Pedro Galán, Alfredo Nicieza, Pedro Tarraso: Understanding hybrid zones between divergent phenotypic and genetic populations of fire salamanders | Richard P.J.H. Struijk: The Amur rat snake (<i>Elaphe schrenckii</i>) in the Netherlands. Distribution, habitat use and reproduction. |
| 10:40 – 11:10 | Coffee Break | |
| 11:10 – 12:30 | Session4c: Phylogeography of European Reptiles & Amphibians Chair: Sylvain Ursenbacher | Session 6a: Ecology & Behaviour of Reptiles Chair: Claudia Corti |
| 11:10 – 11:30 | Carolin Kindler, Maxime Chèvre, Sylvain Ursenbacher, Wolfgang Böhme, Axel Hille, Daniel Jablonski, Melita Vamberger & Uwe Fritz: Hybridization patterns in two contact zones of grass snakes reveal a new Central European snake species | Konrad Mebert, Luca Luiselli, Marco Zuffi, Fabio Petrozzi, Giovanni Amori: Snakes on Montecristo Island, Italy: Dwarfism and resource partitioning |
| 11:30 – 11:50 | Emmanouela Karameta, Petros Lymberakis, Heinz Grillitsch, Çetin Ilgaz, Spyros Sfenthourakis, Panayiotis Pafilis, Nikos Poulakakis: Phylogeny and phylogeography of the Roughtail Rock Agama <i>Stellagama stellio</i> | Katja Schmölz, Adrian Pinder, Gerald Kuchling, Günter Gollmann: Assisted colonization of juvenile Western Swamp tortoise <i>Pseudemydura umbrina</i> into novel wetlands: macro invertebrate communities, biomass and turtle diet |
| 11:50 – 12:10 | Sarita Mahtani-Williams, Bálint Halpern, Judit Vörös, Boris Lauš, Gergely Babocsay, Balázs Vági, Attila László Péntek, Zoltán T. Nagy, Pamela A. Burger: Phylogeography and adaptive evolution of the Caspian whipsnake (<i>Dolichophis caspius</i>) | Dollion, A. Y., G. J. Measey, R. Cornette, L. Carne, K. A. Tolley, J. M. daSilva, R. Boistel, A-C Fabre & A. Herrel: Chameleons, from the genus <i>Bradypodion</i> , are what they eat... with some gender inequalities |
| 12:10 – 12:30 | Vamberger Melita, Stuckas Heiko & Fritz Uwe: Gene flow and unexpected ancient divergences in Western Palearctic pond turtles (<i>Emys</i> spp.) | Ana Golubović: Antipredator response and agility in terrestrial and aquatic Chelonians – steady as a rock or running back to water |
| 12:30 – 13:45 | Lunch Break | |

Wednesday, September 20th – Afternoon

| | Room 1 | Room 2 |
|----------------------|--|---|
| 13:45 – 15:05 | Session 7a: Phylogeny, Taxonomy & Systematics Chair: Judit Vörös | Session 6b: Ecology & Behaviour of Reptiles Chair: Claudia Corti |
| 13:45 – 14:05 | Nikolaus Stümpel, Mehdi Rajabizadeh, Aziz Avci, Wolfgang Wüster, Ulrich Joger: Phylogeny and diversification of mountain vipers (<i>Montivipera</i> , Nilson et al. 2001) in the Near and Middle East | Barry P. Stephenson, Zeshan Velani & Nikolett Ihász: Do albino snakes experience higher rates of attack from avian predators than wild-type snakes? – An experimental test using plasticine models |
| 14:05 – 14:25 | Aaron M. Bauer, Ishan Agarwal and Luis M. P. Ceriaco: Discovering Hidden Diversity in the <i>Hemidactylus</i> (Reptilia: Gekkonidae) of Africa | A.M. Prieto-Ramirez, D. Rödder, G. Pe'er, K. Henle: Realized niche and microhabitat selection of the eastern green lizard (<i>Lacerta viridis</i>) at the core and periphery of its distribution range |
| 14:25 – 14:45 | Luis M. P. Ceriaco & Aaron M. Bauer: Nomenclature and taxonomic status of the genus <i>Blanus</i> Wagler, 1830 (Squamata: Amphisbaenidae) from the Iberian Peninsula | Panayiotis Pafilis, Colin Donihue, Anthony Herrel, Grigoris Kapsalas, Menelia Vassilopoulou-Kampitsi, Anne-Claire Fabre, Johannes Foufopoulos: Habitat and not heredity shape the thermoregulation of a Mediterranean lizard |
| 14:45 – 15:05 | Michael F. Bates & Edward L. Stanley: A new species of dragon lizard from Swaziland and South Africa | Valentin Mingo, Stefan Lötters, Norman Wagner: Impact of pesticide applications and land use intensity on enzymatic activity and fitness of reptiles |
| 15:05 – 15:35 | Coffee Break | |
| 15:35 – 16:55 | Session 7b: Phylogeny, Taxonomy & Systematics Chair: Sylvain Ursenbacher | Session 8a: Ecology & Behaviour of Amphibians Chair: Walter Hödl |
| 15:35 – 15:55 | Michael Veith, Sarah Kieren: The changing views on the evolutionary relationships of extant Salamandridae (Amphibia: Urodela) | Daniela C. Röbber, Marcelo Menin, Albertina P. Lima, Heike Pröhl, Stefan Lötters: A new type of aposematism? Flashy feet in Neotropical harlequin toads (<i>Atelopus</i>) are a conspicuous signal to predators |
| 15:55 – 16:15 | Šunje E, Van Damme Raoul, Jelić D, Muller M, Škrijelj, R & Helfer, V.: Morphometric characteristics of alpine salamanders; a support for subtaxonomy and conservation? | Cogălniceanu Dan, Plăiașu Rodica, Stănescu Florina, Băncilă Raluca, Ruști Dorel: Out in the cold: adaptive strategies of the Common Frog (<i>Rana temporaria</i>) in extreme habitats |
| 16:15 – 16:35 | Morten E. Allentoft, Arne Redsted, Hans Viborg Kristensen: Damaged by time and formalin: Using ancient DNA technology to recover century-old snake mitogenomes | Sebastiano Salvidio, Federico Crovetto, Fabrizio Oneto, Giulia Palumbi, Antonio Romano, Andrea Costa: The use of clay models to assess potential predation on cave salamanders |
| 16:35 – 16:55 | Evgeniy Simonov, Daniel Melnikov, Daniel Jablonski, Artem Kidov, Nikolay Orlov: Who is who in <i>Gloydus halys-intermedius</i> complex? New insights from extended geographical sampling and consequences for venom biochemistry research | Carolyn Dittrich, Ariel Rodríguez, Ori Segev, Sanja Drakulić, Heike Feldhaar, Miguel Vences & Mark-Oliver Rödel: Temporal migration patterns and mating tactics influence size-assortative mating in <i>Rana temporaria</i> |
| 17:15 – 19:00 | SEH Ordinary General Meeting | |

Thursday, September 21st – Morning

| | | | |
|---------------|---|--|---|
| 08:30 – 09:15 | Plenary Lecture: Mathieu Denoel: Facultative paedomorphosis in pond breeding newts Chair: Eva Ringle | | |
| | Room 1 | Room 2 | Room 3 |
| 09:20 – 10:40 | Session 6c: Ecology & Behaviour of Reptiles Chair: Jelka Crnobrnja-Isailovic | Session 9a: Diseases & Anomalies Chair: Florian Glaser | Micro CT Workshop 1 Chair: Chris Broeckhoven |
| 09:20 – 09:40 | Laurane Winandy, Lucie Di Gesu, Marion Lemoine, Staffan Jacob & Julien Cote: Conspicuous attraction in a landscape of fear: How different sources of information about predation risk affect sociability in lizard | Stefano Canessa, Claudio Bozzuto, Evan H. Campbell Grant, Sam S. Cruickshank, Matthew C. Fisher, Jacob C. Koella, Stefan Lötters, An Martel, Frank Pasmans, Ben C. Scheele, Annemarieke Spitzen-van der Sluijs, Sebastian Steinfartz, Benedikt R. Schmidt: Finding evidence-based amphibian conservation strategies in an emergency | Keynote: Chris Broeckhoven & Anton du Plessis: Micro-computed tomography (micro-CT) in herpetological research: concepts, practical considerations and potential |
| 09:40 – 10:00 | Bianca M. op den Brouw, Syed A. Ali, Nicholas R. Casewell, Behzad Fathina, Parviz Ghezellou, Bryan G. Fry: Functional variation in the venom of desert vipers <i>Pseudocerastes</i> and <i>Eristicophis</i> (Viperinae: Viperidae) | Blooi, Mark, Laking, Alexandra E., Martel, An, Haesebrouck, Freddy, Jocque, Merlijn, Brown, Tom, Green, Stephen, Vences, Miguel, Bletz, Molly C., Frank Pasmans: Host Niche May Determine Disease-Driven Extinction Risk | Chris Broeckhoven, Celeste De Kock & P. le Fras N. Mouton: Sexual dimorphism in osteoderm expression and the role of male intrasexual aggression |
| 10:00 – 10:20 | Verónica Gomes, Antígoni Kalliontzopoulou, Miguel A. Carretero & Anthony Herrel: Morphological trade-offs between duration and maximal capacity of bite performance in <i>Podarcis bocagei</i> | Annemarieke Spitzen – van der Sluijs, Stefano Canessa, Mark Blooi, An Martel & Frank Pasmans: The long-term cost of chytridiomycosis for yellow-bellied toads | Peter Pogoda & Alexander Kupfer: Flesh and bone: an integrative approach towards sexual size dimorphism of spectacled salamanders |
| 10:20 – 10:40 | Pantelis Savvides, Eleni Georgiou, Panayiotis Pafilis, Spyros Sfenhourakis: Ontogeny and differential substrate use among populations of the spiny footed lizard (<i>Acanthodactylus schreiberi</i>): Plasticity or an ongoing evolutionary adjustment? | János Ujszegi, Tamás Drexler, Márk Z. Németh, Judit Vörös, Ágnes M. Mőricz, Dániel Krőszelyi, Attila Hettyey: Investigating the effects of infection with <i>Batrachochytrium dendrobatidis</i> on life history traits and skin toxin production of <i>Bufo bufo</i> tadpoles | Mark D. Scherz, Hervé Philippe, Andolalao Rakotoarison, Frank Glaw, & Miguel Vences: Micro-CT, morphometrics, and phylotranscriptomics shed light on the underpinnings of convergent miniaturization in a radiation of frogs |
| 10:40 – 11:10 | Coffee Break | | |
| 11:10 – 12:30 | Session 8b: Ecology & Behaviour of Amphibians Chair: Dan Cogalniceanu | Session 9b: Diseases & Anomalies Chair: Annemarieke Spitzen-Van der Sluijs | Micro CT Workshop 2 Chair: Chris Broeckhoven |
| 11:10 – 11:30 | Frank Pasmans, Elin Verbrugge, Max Sparreboom, Feng Xie, An Martel: Adaptations of the amphibian egg and hatchling for terrestrial nesting in the Wenxian newt (<i>Tylotriton wenxianensis</i>) | Kärverno S., Meurling, S., Laurila, A. & J. Höglund: Revealing high-risk ponds of the chytrid fungus in Swedish amphibian populations | Claudia Koch & Nadine Schwarz: Diversity of South American wormsnares of the genus <i>Epictia</i> : Analysis of internal morphological characters via Micro-CT images |
| 11:30 – 11:50 | Kathleen Preißler & Heike Pröhli: The effects of background coloration and dark spots on the risk of predation in poison frog models | Elena A. Kulikova, Vojtech Baláz, Mihails Pupins, Aija Pupina: The first records of <i>Batrachochytrium dendrobatidis</i> in Latvian Zoo | Practical Workshop |
| 11:50 – 12:10 | Sergey M. Lyapkov: Amphibian adaptation to extreme environment in statu nascendi: water frogs and brown frogs in cold climatic conditions | R.G. Bina Perl, Sarig Gafny, Eli Geffen, Miguel Vences: Detection of <i>Bd</i> and exploration of microbial communities of amphibians in northern Israel | Practical Workshop |
| 12:10 – 12:30 | | Klaus Henle: A global database of anomalies in natural populations of amphibians – and global patterns derived from the database | Practical Workshop |
| 12:30 – 13:45 | Lunch Break | | |

Thursday, September 21st – Afternoon

| | Room 1 | Room 2 |
|----------------------|---|--|
| 13:45 – 15:05 | Session 8c: Ecology & Behaviour of Amphibians Chair: Günter Gollmann | Session 10a: Symposion Bsal Chair: Stefan Lötters |
| 13:45 – 14:05 | Eliane Küpfer, Daniel Goebloed, Ori Segev, Leon Blaustein, E. Tobias Krause and Sebastian Steinfartz: Mechanisms to avoid fish as predators in stream side salamander larvae (<i>Salamandra infraimmaculata</i>) | Alex Laking, Hai Ngoc Ngo, Frank Pasmans, An Martel & Tao Thien Nguyen: <i>Batrachochytrium salamandrivorans</i> is the predominant chytrid fungus in Vietnamese salamanders |
| 14:05 – 14:25 | Elnaz Najafi Majid, Elif Yildirim, Uğur Kaya: Altitudinal variation in body size and age structure of the Urmia Lake Newt, <i>Neurergus crocatus</i> Cope, 1862 in Iraq | Florian Glaser, Markus Grabher, Gerda Ludwig, Gopi Munimanda, Gernot Pechlaner, Peter Kaufmann, Silke Schweiger, Steve Smith, Karina Smole-Wiener, Thomas Wampula, Anton Weissenbacher, Chris Walzer & Doris Preininger: <i>Batrachochytrium salamandrivorans</i> Monitoring in Austria - experiences from Lake Constance to Vienna |
| 14:25 – 14:45 | Andrea Meloto, Gentile Francesco Ficetola, Raoul Manenti: To bite or not to bite? Cannibalism ecology in different fire salamander populations | Stefan Lötters, Lutz Dalbeck, Heidrun Düssel-Siebert, Kai Kirst, An Martel, Dagmar Ohlhoff, Frank Pasmans, Sebastian Steinfartz, Miguel Vences, Norman Wagner, Josef Wegge, Michael Veith: The status of <i>Batrachochytrium salamandrivorans</i> in its potential center of dispersal in the Eifel, Germany |
| 14:45 – 15:05 | Manenti Raoul, Enrico Lunghi, Claudia Canedoli, Roberta Pennati, Giorgio Scari, Roberta Cimmaruta & Gentile Francesco Ficetola: Integrating micro-scale analyses in the study of niche similarity: preliminary results in the hybrid zone between <i>Hydromantes italicus</i> and <i>H. Ambrosii</i> | Gwij Stegen, Annemarieke Spitzen-van der Sluijs, Sergé Bogaerts, Stefano Canessa, Sebastian Steinfartz, Ralf Hendrix, Nico Janssen, Wilbert Bosman, Frank Pasmans & An Martel: Conservation by fragmentation: a case study using a recently discovered fire salamander (<i>Salamandra salamandra</i>) population near the index outbreak site, free of <i>B. salamandrivorans</i> |
| 15:05 – 15:35 | Coffee Break | |
| 15:35 – 17:15 | Session 11: Biogeography, Diversity & Distribution Chair: Antigoni Kaliontzopoulou | Session 10b: Symposion Bsal Chair: Stefan Lötters |
| 15:35 – 15:55 | Karin Ernst, Günter Gollmann, Christoph Plutzer & Silke Schweiger: Species distribution modeling and field survey on <i>Iberolacerta horvathi</i> (Méhely, 1904) in Austria: predicted and actual habitats considering topographical, geological and bioclimatic parameters | Wouter Beukema, An Martel, Tao Thien Nguyen, Koichi Goka, Dirk S. Schmeller, Zhiyong Yuan, Alexandra E. Laking, Truong Quang Nguyen, Chun-Fu Lin, Jennifer Shelton, Adeline Loyau & Frank Pasmans: Colonisation and spread of <i>Batrachochytrium salamandrivorans</i> in the Western Palearctic is governed by changes in pathogen niche structure and environmental context |
| 15:55 – 16:15 | Arthur Tiutenko: New distributional records for the herpetofauna in the southern Ethiopian Highlands | Hannah Keely Smith, Frank Pasmans & An Martel: Differential response of <i>Batrachochytrium salamandrivorans</i> to salamander mucosomes reflects species susceptibility |
| 16:15-1 6:35 | Florian Glaser, Michael Haupolter, Gerda Ludwig: Distribution and ecology of the Fire Salamander in Tyrol - notes from the regional range boundary within the Alps | Dirk Schmeller, Alan Chan-Alvarado, Lin Chun-Fu, Adeline Loyau, Jennifer Sheldon, Vance Vredenburg: The microbiome of rare Taiwanese salamanders and their infection status |
| 16:35 – 16:55 | Omar Torres Carvajal: Reptiles from the Tropical Andes: A decade of wonderful discoveries | Molly Bletz, Moira Kelly, Miguel Vences, Sebastian Steinfartz, Emma Bales, Joana Sabino Pinto, An Martel & Frank Pasmans: Friend or Foe: the role of bacteria in <i>Bsal</i> infection of Fire Salamanders |
| 16:55 – 17:15 | Mariana P. Marques, Luis M. P. Ceriaco, David C. Blackburn & Aaron M. Bauer: Diversity and Distribution of the Amphibians and Reptiles of Angola: Turning historical records into an Atlas of the Angolan herpetofauna | Joana Sabino-Pinto, Miguel Vences, Sebastian Steinfartz: Screening of the fungal pathogens <i>Batrachochytrium dendrobatidis</i> and <i>B. salamandrivorans</i> (<i>Bsal</i>) in captivity indicates asymptomatic infections and clearance of <i>Bsal</i> through heat treatments |
| 17:15 – 18:00 | Poster Session 2 | |

Friday, September 22nd – Morning

| | | |
|----------------------|--|--|
| 08:30 – 09:15 | Plenary Lecture: Maria Schindler: Surviving in a semi-natural environment – 20 years of European Pond Turtle conservation in the Donau-Auen National Park Chair: Andreas Maletzky | |
| | Room 1 | Room 2 |
| 09:20 – 10:40 | Session 12a: Practical Conservation Chair: Katja Pobeljšaj | Session 13a: Evolution & Development Chair: Miguel Vences |
| 09:20 – 09:40 | Gregor Lipovšek, Urban Dajčman, Katarina Drašler, Melita Vamberger, Miha Krofel, Anamarija Žagar: Reproductive success of the European pond turtle (<i>Emys orbicularis</i>) on Ljubljana marshland | Günter Schultschik, Silke Schweiger, Christa Mayer, Georg Gassner, Günter Gollmann: Data fabrication by Paul Kammerer: the testimony of Franz Megusar |
| 09:40 – 10:00 | Uğur Kaya, Elif Yilirim, Elnaz Najafi Majd, Müge Gidiş, H. Bradley Shaffer, Garry Bucciarelli, Ben Wielstra, Kevin Neal: Let's save the amphibians, but how? A case conservation study of <i>Lyciasalamandra</i> species | Martina Gregorovicova, Alena Kvasilova, Jermo Hanemajler, Bjarke Jensen, Vincent Christoffels & David Sedmera: A window to the heart development of Squamata: cardiac conduction system and level of ventricular septation |
| 10:00 – 10:20 | Elin Soomets, Asko Lõhmus & Riinu Rannap: Brushwood removal from ditch banks attracts breeding frogs in drained forests | Alexander Kupfer, Erin Maxwell, Sandy Reinhard & Susanne Kuehnel: The evolution of parental investment in caecilian amphibians |
| 10:20 – 10:40 | Bálint Halpern, Tamás Péchy, Tibor Somlai, Róbert Dankovics, Endre Sós, Chris Walzer: Survival, area use and thermoregulation of reintroduced Hungarian meadow vipers (<i>Vipera ursinii rakosiensis</i>) | Eugenia Sanchez, Eliane Kúpfer, Daniel J. Goedbloed, Arne W. Nolte, Tim Lüddecke, Stefan Schulz, Miguel Vences, Sebastian Steinfartz: Development of the fire salamander: morphology and gene expression similarities to anurans |
| 10:40 – 11:10 | Coffee Break | |
| 11:10 – 12:30 | Session 12b: Practical Conservation Chair: Tony Gent | Session 13b: Evolution & Development Chair: Miguel Vences |
| 11:10 – 11:30 | Mohammed Znari & Nawal Hichami: Demographic decline and population viability analysis of the Souss valley tortoise (<i>Testudo graeca soussensis</i>) in a degraded arid steppe-land of west-central Morocco: implications for conservation and management | Constantijn Raaymakers, Elin Verbrugge, Sophie Hernot, Tom Hellebuyck, Cecilia Betti, Cindy Peleman, Myriam Claeys, Wim Bert, Vicky Cavelliers, Steven Ballet, An Martel, Frank Pasmans & Kim Roelants: A molecular toxin delivery system in amphibians |
| 11:30 – 11:50 | Konrad Mebert, Bayram Göçmen, Naşit İğci, Mehmet Anil Oğuz, Mert Kariş: Anatolian Vipers - comparing IUCN Red List Data and state of knowledge: <i>Vipera eriwanensis</i> , <i>V. davevskii</i> , <i>V. anatolica</i> , <i>Montivipera (bulgardaghica) albizona</i> | Kim Roelants, Constantijn Raaymakers, Lumeng Ye, Benoit Stijlemans, Pierre Cornelis, An Martel: Tracing the evolutionary rise of an amphibian molecular defence arsenal by resurrecting ancestral skin peptides |
| 11:50 – 12:10 | Georgi Popgeorgiev, Dimitar Plachiyiski, Yurii Kornilev & Nikolay Tzankov: Land-management subsidies for shrub removal are a major threat for herpetofauna in Bulgaria | Lilijana Bizjak Mali and Stanley K. Sessions: Explanation for Reproductive Abnormalities in the European Blind Cave Salamander, <i>Proteus anguinus</i> |
| 12:10 – 12:30 | David O'Brien, J.E. Hall A. Miró, CT O'Brien, J. McKinnell, T. Seymour & R. Jehle: Coming together to conserve a species at the edge of its range: <i>Triturus cristatus</i> in the Scottish Highlands | Stanley K. Sessions and Lilijana Bizjak Mali: Evolutionary Cytogenetics of Proteid Salamanders |
| 12:30 – 13:45 | Lunch Break | |

Friday, September 22nd – Afternoon

| | Room 1 | Room 2 |
|----------------------|--|--|
| 13:45 – 15:25 | Session 12c: Practical Conservation Chair: Andreas Maletzky | Session 13c: Evolution & Development Chair: Sebastian Steinfartz |
| 13:45 – 14:05 | Tony Gent, Jim Foster and John W. Wilkinson: Spatial planning for herpetofauna conservation: developing and using metrics for setting goals and taking forward action | Anushree Jadhav: Comparative study of anuran skeletal development in <i>Duttaphrynus melanostictus</i> (Bufonidae), <i>Fejervarya</i> <i>syhadrensis</i> (Dicroglossidae) and <i>Microhyla ornata</i> (Microhylidae) during metamorphosis. |
| 14:05 – 14:25 | Martin Kyek, Peter Kaufmann, Robert Lindner: Long term population trends of <i>Bufo bufo</i> and <i>Rana</i> <i>temporaria</i> : An analysis of two decades amphibian fencing in Salzburg | Życzyński A., Kaczmarek A., Nowak Z.: Initial study on polymorphism of sex linked gene CHD in Serpentes suborder |
| 14:25 – 14:45 | Benjamin Lejeune, Gilles Lepoint, Mathieu Denoël: Complex impact of goldfish introduction on palmate newt dominated pond ecosystem | Magdalena Kowalska, Weronika Rupik: How does the pancreas of snakes differentiate? |
| 14:45 – 15:05 | Schäfer A.M.: Informed conservation management on yellow-bellied toads; influence of different land use and succession on population dynamics at a former military area | Mateusz Hermyt, Katarzyna Janiszewska, Weronika Rupik: Structure and ultrastructure of the developing egg tooth in squamate reptiles |
| 15:05 – 15:25 | Silviu O. Petrovan: Amphibians, roads and evidence-based conservation | Paweł Kaczmarek, Katarzyna Janiszewska, Weronika Rupik: Embryology of the vomeronasal organ and associated structures in the brown anole (Squamata: Iguania) |
| 15:25 – 15:55 | Coffee Break | |
| 16:00 – 17:00 | Council Meeting 2 | |
| 17:15 – 17:45 | Closing Ceremony | |

PLENARY LECTURES

Facultative paedomorphosis in pond-breeding newts

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During their life cycle, pond-breeding newts usually undergo a metamorphosis that makes the transition between the aquatic gilled larval stage and the terrestrial metamorphosed stage. One of the characteristics of this shift is the resorption of the gills and the closure of gill slits. However, in some populations, whereas some individuals metamorphose, others retain their larval features such as gills while acquiring sexual maturity, a pattern known as facultative paedomorphosis. Although rarer than metamorphosis, paedomorphosis is expressed in numerous species and populations, making it not anecdotic. It is therefore thought to play an important role in ecology and evolution.

Facultative paedomorphosis is a polyphenism in which alternative phenotypes are produced in response to environmental variables but is also clustered in some geographic areas. Although paedomorphs are mainly found in deep and fishless habitats, such as alpine lakes, they can also be present in semi-permanent ponds. By being able to detect changes of aquatic conditions, individuals can shift tactic, i.e. paedomorphs can transform into the dispersal phenotype, the metamorph. Being paedomorphs in such risky conditions remains advantageous as it allows an early reproduction (i.e. progenesis) compared with the metamorphs. Although there are sex effects at varied levels, males and females respond to environmental change and both morphs are sexually active. This translates also in a large gene flow across phenotypes within ponds. Such an absence of sexual isolation allows the persistence of both developmental strategies in heterogeneous environments and is therefore contrasting with patterns of obligate paedomorphosis which are found in some salamanders.

Coexisting paedomorphs and metamorphs exhibit temporal, spatial and food resource partitioning, but with variations across populations. Temporal partitioning occurs in sites where metamorphs leave water for land during a part of the year whereas micro-habitat and food specializations are found during their aquatic cohabitation. Their different trophic morphology can explain the different diet patterns but also their preferences for different micro-habitats. This suggests that facultative paedomorphosis can be considered to be a trophic polyphenism and that its selection is not only caused by the advantages of life in water versus on land, but also through the heterogeneity within aquatic environments.

The low number of populations of paedomorphs and their continuous use of the aquatic habitats make them very vulnerable. Longitudinal surveys show they are declining at a very

high rate across all their distribution range. For instance, the most remarkable populations of paedomorphs, previously known as subspecies in the Balkans are all gone. The main driver of this decline is the introduction of alien species in ponds and lakes, extirpating first paedomorphs and then metamorphs. The only hope is that, being a polyphenism, paedomorphosis shows fast resilience in some populations. Drought could therefore help removing fish but may also be a disaster. On another hand, because of a potential long-lasting counter-selection against paedomorphosis in some populations, specifically in alpine lakes, it is likely too late. Conservation actions should therefore take place to remove threats and protect the main populations of paedomorphs as highly valuable part of intraspecific diversity.

References

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Herpetofaunal diversity and endemism in Indochina: new discoveries and biogeographic patterns

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Indochina has long been recognized as a major evolutionary hotspot. The region cradles one of the world's richest herpetofauna: the Eastern Indochina (Vietnam, Cambodia and Laos) together with Thailand hosts around 384 species of amphibians and 734 species of reptiles. The species diversity of amphibians and reptiles has been remarkably increasing, and the significant portion of this diversity was discovered only within the last 20 years. E.g., in Vietnam the number of recorded species of amphibians increased almost three folds from 82 to 244 species (298%) and of reptiles almost two folds from 258 to 443 species (172%). Despite this remarkable progress, our knowledge on herpetofaunal diversity of the Indochinese region remains insufficient. The general understanding of Indochina's herpetofaunal biogeography and its origins is also hampered by (1) uneven knowledge and surveying efforts on herpetofauna of different areas; (2) high speed of new data accumulation; (3) long-term anthropogenic modification of habitats; (4) absence of a wide-scale phylogenetically based analysis of species diversity and endemism patterns in Indochina.

On the coming opportunity, I would like to review research results of my colleagues and myself, with special reference to contributions of morphological, bioacoustic and especially of molecular data. I will argue that the main factors shaping herpetodiversity of Indochina are:

(1) Long and complex geological and climatic history of the Indochinese landmass, led to a high percent of *in situ* diversification. Within-area diversification and subsequent emigration appear to play a key role in formation of Indochinese herpetofauna since at least the early Miocene. Some striking examples of highly divergent relic lineages of amphibians and reptiles will support this statement.

2) Geographic location of Indochina, lying on the cross-roads between Eastern Asia, Indian subcontinent and Sundaland, facilitated faunal exchange between these areas. Cycles of connection and isolation between Mainland Asia and Sundaland allowed dispersal events from Indochina to Borneo and Malaya and *vice versa*. The Indian collision also led to multiple faunal exchanges between Indochina and Indian subcontinent, which went in both directions. Several groups of amphibians and reptiles show examples of dispersal between Indochina and Australasia.

3) Mountain areas of Indochina, in particular the Annamites and the Cardamoms, represent independent areas of elevated (micro)endemism. Isolated montane forest islands enabled diversification through vicariance; it appears that cases of long-distance dispersal from other forest islands were quite limited. Offshore islands of Indochina also played an important role in shaping regional herpetodiversity. Another fascinating example of high local endemism is connected with karst areas of northern Vietnam and Laos.

4) The major river basins of Indochina (Mekong, Chao Phraya and Red River) act as an important zoogeographic barriers for both regional faunas and on the level of cryptic species complexes. Some examples of molecular and bioacoustic analyses will illustrate this.

5) High diversity of climatic conditions in Indochina led to ecological specialization in certain groups, which include remarkable examples of diversification through ecological opportunity.

The Indochinese Peninsula is a region with a globally important level of herpetofaunal diversity. Accelerated efforts to conserve Indochinese herpetofauna, currently housing the highest levels of amphibian and reptile species richness, are critically required.

Why to not study poison in poison frogs

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Poison frogs (Dendrobatidae) once started to attract attention to both scientists and hobbyists due to the highly conspicuous appearance and associated high toxicity of certain species. Even the common name of this frog family ‘poison dart frogs’ is derived from the habit of some native tribes that rubbed the secretions of highly toxic species onto their hunting weapons. However, neither all dendrobatid frogs feature high levels of toxicity, nor does this trait represent a synapomorphy of the entire family. It is rather the case that all species of poison frogs are characterized by highly complex social and reproductive behaviour, involving territoriality, female choice, prolonged courtship, and parental care.

I have dedicated most of my research efforts to the dendrobatid frog *Allobates femoralis*, which is non-toxic and non-colourful, but has proven to be an optimal model species for studying poison frog mating and parental behaviour. Recently, we have established an experimental population of *A. femoralis* on a river island by the controlled introduction of 1800 morphologically measured and genetically sampled tadpoles. At the same time we set up a captive breeding population currently housed at the University of Vienna. The possibility to study these frogs in the field in a closed system as well as to perform experiments under controlled laboratory conditions allows us to address a wide array of research questions, ranging from animal cognition to population ecology and evolution.

In our lab we apply a wide variety of methods to achieve these goals, such as focal observations, individual tracking, molecular parentage analysis and video monitoring. Our most recent findings show that these little forest frogs are very thoughtful parents, exhibit excellent navigation and orientation abilities, as well as behavioural flexibility across various contexts. By comparing these findings to results from other dendrobatid species and across animal taxa, we aim to identify and better understand selective forces that shape reproductive and parental behaviours in animals at both the individual as well as the population level.

References

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Surviving in a semi-natural environment – 20 years of European Pond Turtle conservation in the Donau-Auen National Park

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In Austria the habitat of the European Pond Turtle (*Emys orbicularis*), the only native turtle species of this region, has been restricted to the riverine landscape of the Donau-Auen National Park. Some 2.000 turtles inhabit the floodplains east of Vienna and form one of the largest reproductive populations in central Europe, the only one in Austria.

Starting with the first regulation of Danube River, almost 150 years ago, large-scale changes influenced the floodplains. Today the Pond Turtle population depends on both natural and man-made habitats, resulting in conflicting management options. In the immediate vicinity of Vienna “modern” impacts like introduction of allochthonous turtles and disturbance by increasing outdoor activities become important more and more.

A species conservation program, initiated by the Nationalpark in 1997 combines research, conservation and public awareness with the aim to realize a management program with minimized intervention and precise support for the remaining autochthonous population.

Results of genetic analysis, telemetric observations, phaenology of reproductive behaviour, and findings on reproductive success will be presented and discussed in terms of its significance for species conservation and habitat management within a national park.

BATRACHOCHYTRIUM SALAMANDRIVORANS (BSAL)
SYMPOSION

Colonisation and spread of *Batrachochytrium salamandrivorans* in the Western Palearctic is governed by changes in pathogen niche structure and environmental context

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Identifying hosts and regions susceptible to invasion by an emerged pathogen is vital to inform early risk assessments. Although ecological niche modelling is often used for this aim, it remains unknown whether measurements of pathogen-host niche overlap or spatial predictions of disease spread are affected if a pathogen's niche changes during invasion, or when environmental conditions in native and invasive ranges differ. We explored these issues using the recent emergence of *Batrachochytrium salamandrivorans* (*Bsal*) as a critical, empirical example.

To build ecological niches we first gathered occurrences from the native Asian and invasive European distribution of *Bsal*. Through ordination in PCA-bound environmental space we then applied overlap tests to compare native and invasive *Bsal* niches with those of 56 putative WP host species. Finally, we ensembled bivariate niche models (ESMs) for each *Bsal* niche to estimate potential further spread across the Western Palearctic (WP). Our results revealed that the invasive *Bsal* niche is a conservative, unfilled subset of its wider native niche. Pathogen-host overlap measured using the narrow invasive niche was nevertheless found to

be significantly higher than that of the native niche, which is partially situated in subtropical conditions absent from the WP. ESMS created using the native niche predict moderate to high suitability for *Bsal* throughout Europe.

Conversely, a restricted but highly suitable range was predicted using the invasive niche, which coincides with presence of oceanic climates in north-western Europe and several Mediterranean mountain ranges. We conclude that unequal relative availability of environments in native and invasive ranges may lead to underestimation of niche overlap between native pathogen populations and putative hosts in the invasive range. Niche unfilling might subsequently add uncertainty to overlap measurements, and predictions of potential further spread. Results based on the invasive niche therefore provide a conservative estimate; yet demonstrate, in our case, that *Bsal* is already present in conditions shared by numerous host species. Further niche filling may accordingly increase risk for salamander diversity across the WP.

Friend or Foe: the role of bacteria in Bsal infection of Fire Salamanders

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The Fire Salamander, *Salamandra salamandra*, is highly susceptible to the pathogen, *Batrachochytrium salamandrivorans* (*Bsal*), with *Bsal*-induced chytridiomycosis causing rapid population declines. Skin microbiota have been documented to play an important role in *B. dendrobatidis*-infection dynamics; therefore, it can be hypothesized that microbiota may play a role in *Bsal*-fire salamander infection dynamics.

We investigated the microbiota on wild healthy salamanders as well as the response of salamander skin microbiota to *Bsal* infection and the potential of adding cultured *Bsal*-inhibiting bacteria to salamander skin to alter infection dynamics. We found that fire salamanders maintain microbes on their skin, however, their density is low, averaging 4.03×10^4 cell equivalents per adult salamander.

Experimental infection of fire salamanders had no effect on skin bacterial richness, community structure or abundance; however, did result in septicemic events via opportunistic pathogens, likely resulting from the breaching of the skin by *Bsal*. Furthermore, we found that resident bacteria exhibited a range of functional capacities against *Bsal*, from inhibition to enhancement of *Bsal* growth. The addition of high concentrations of a cultured bacterium to fire salamander skin resulted in a marginal increase in time to death, with one individual surviving through the experiment.

These results suggest that the microbiota living on fire salamander skin may not provide sufficient protection against *Bsal* perhaps due to low bacterial numbers combined with *Bsal*'s ability to disseminate inside salamander skin, thus averting bacterial defenses. However, the survival of one bacterial-treated individual leaves open the possibility of probiotic disease mitigation.

***Batrachochytrium salamandrivorans* Monitoring in Austria - experiences from Lake Constance to Vienna**

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In spring 2016, monitoring of *Batrachochytrium salamandrivorans* (*Bsal*) infection in wild populations of *Salamandra salamandra* in Vienna and Tyrol (Austria) as well as in selected captive collections (Alpenzoo Innsbruck, Tiergarten Schönbrunn, main private collections) was started. In Tyrol also *Ichthyosaura alpestris* and *Lissotriton vulgaris* were tested.

All 335 samples (55 ind. from captive collections, 280 ind. from the wild) of 2016 were *Bsal* negative according to the DNA-based assay. In 2017 the monitoring project was continued in Tyrol and Vienna furthermore first investigations took place in the provinces of Salzburg, Lower Austria, Carinthia and Vorarlberg, facilitated by voluntary engagement of local herpetologists. In Tyrol, besides *Salamandra salamandra* and *Ichthyosaura alpestris*, selected populations of *Salamandra atra* were checked for *Bsal*-infection for the first time. Additionally, *Cynops* spp. from pet trade and specimens of *Triturus dobrogicus*, *Lissotriton vulgaris* and *Ichthyosaura alpestris* from the Alpenzoo Innsbruck were investigated.

We report on the current status of *Bsal* in Austria and furthermore discuss recommendations for national and international management.

***Batrachochytrium salamandrivorans* is the predominant chytrid fungus in Vietnamese salamanders**

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The amphibian chytrid fungi, *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*), pose a major threat to amphibian biodiversity (SKERRATT et al. 2007; FISHER et al. 2012; MARTEL et al. 2014). Recent evidence suggests Southeast Asia as a potential cradle for both fungi, which likely resulted in widespread host-pathogen co-existence (MARTEL et al. 2014). To examine this we sampled 583 salamanders from 8 species across Vietnam in 55 locations for *Bsal* and *Bd*, determined scaled mass index as a proxy for fitness and collected environmental data. *Bsal* was found within 14 of the 55 habitats (2 of which it was detected in 2013), in five salamandrid species, with a prevalence of 2.92%. The globalized pandemic lineage of *Bd* was found within one pond on one species with a prevalence of 0.69%. Combined with a complete lack of correlation between infection and individual body condition (Spearman's correlations < ±0.18) and absence of indication of associated disease, this suggests low level pathogen endemism and *Bsal* and *Bd* co-existence with Vietnamese salamandrid populations. *Bsal* was more widespread than *Bd*, and occurs at temperatures higher than tolerated by the type strain, suggesting a wider thermal niche than currently known. Therefore, this study provides support for the hypothesis that these chytrid fungi may be endemic to Asia and that species within this region may act as a disease reservoir.

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The status of *Batrachochytrium salamandrivorans* in its potential center of dispersal in the Eifel, Germany

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Batrachochytrium salamandrivorans (*Bsal*) is parasitic skin fungus causing chytridiomycosis in caudate amphibians. Native to Asia, it has apparently become invasive in Europe and poses a serious threat to western Palearctic amphibian diversity. Especially the fire salamander, *Salamandra salamandra*, suffers from the effects of this pathogen. While European newt species are sometimes able to clear infection, *Bsal* leads to dramatic decline and (likely) extinction of local fire salamander populations. In its non-native range, this emerging infectious disease is currently known in the wild from Belgium, Germany and the Netherlands. Most records are in the Ardennes and the Eifel. The first record of *Bsal* in Germany (Eifel) was in 2015, shortly after we had become aware of its existence at nearby sites in Belgium and the Netherlands. Up to 2017, this pathogen has been recorded at six sites in the Eifel. Some of these are apparently recent outbreaks, prior to which *Bsal* is expected to not have been there. However, for some years now, we also witness remarkable declines and absences of fire salamander populations in the general area. Moreover, preserved specimens collected more than 10 years ago show signs of *Bsal*-caused chytridiomycosis, indicating that *Bsal* has been in the area for a relative long time and has its center of dispersal in the Eifel.

Screening of the fungal pathogens *Batrachochytrium dendrobatidis* and *B. salamandrivorans* (*Bsal*) in captivity indicates asymptomatic infections and clearance of *Bsal* through heat treatments

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Many factors are threatening amphibian populations, and leading to their declines worldwide, one of them being the emerging infectious disease chytridiomycosis. Two fungi have been associated with the disease, *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*). The latter was recently detected in Netherlands, Belgium and Germany where it drove entire salamander populations to extinction. This pathogen, *Bsal*, has been detected in salamanders from the pet trade (it being likely the pathway through which it reached Europe) and which could still contribute its spread. Eighteen captive collections of amphibians were sampled in Germany and two in Sweden to explore the current extent of *Bsal*. Although *Bsal* associated mortality was only found in one collection, the fungus was detected by quantitative Polymerase Chain Reaction (qPCR) at low amounts in nine collections, without clinical symptoms. Heat treatments were applied in three collections resulting in the complete clearance of the infection on posterior sampling, including the collection that showed *Bsal* associated mortality. We regard the observed *Bsal* positives as true *Bsal* infections, although we cannot exclude that in some cases they could be caused by the probe amplifying other, less virulent *Bsal* strains, or other, unknown chytrid species. The widespread presence of *Bsal* in captive collections is of high concern due to the increased likelihood of leaking the fungus into the wild. Considering the apparent presence of asymptomatic *Bsal* infections, biosafety measures are recommended to be applied to all collections even after heat treatment and when clinical signs are not present.

The microbiome of rare Taiwanese salamanders and their infection status

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Taiwan harbours a large number of amphibian species. Currently, 42 species are described most of which are endemic to this small island (35,883 km²) in the China Sea. In total seven salamander species are known from Taiwan of which all five Hynobiidae salamanders are endemic, rare and restricted to small areas in the mountain range of Taiwan. We sampled three of these species, *Hynobius formosa*, *H. sonani*, and *H. alishani*. We report on their infection status and skin microbiome and elucidate on the importance of the microbiome for the protection of these species.

Differential response of *Batrachochytrium salamandrivorans* to salamander mucosomes reflects species susceptibility

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In Europe, the threat of *Bsal* to salamander populations is demonstrated by the rapid decline of fire salamander populations in Germany, the Netherlands and Belgium (SPITZEN-VAN DER SLUIJS et al. 2013, 2016). Although most European urodelans are susceptible to infection in infection trials (MARTEL et al., 2014), recent evidence suggests marked interspecific differences in the course of infection, with potentially far reaching implications for salamander conservation (STEGEN et al. 2017). As a salamander's skin is the first line of defense against such pathogens, interspecific differences in its innate immune function may at least in part explain differential susceptibility. Here we investigate if compounds present on a salamander's skin can kill *Bsal* spores and if there is variation among species. We used a non-invasive assay to compare killing ability of salamander mucosomes (WOODHAMS et al. 2014) of four different species (*Salamandra salamandra*, *Ichtyosaura alpestris*, *Cynops pyrrhogaster* and *Lissotriton helveticus*) by inoculating *Bsal* zoospores with salamander mucosomes and determining spore survival. In all samples, zoospores were killed when exposed to mucosomes. Moreover, we saw a significant variation in *Bsal* mortality between species, with the highest percent spore survival of 78% in the highly susceptible species *S. salamandra*, and the lowest percent spore survival of 18% in the more resistant *I. alpestris*. Our results indicate that mucosomes of salamanders might provide crucial skin protection against *Bsal*, and could explain why some species are more susceptible than others. This study represents a step towards better understanding species variation in innate immune function and disease susceptibility.

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Conservation by fragmentation: a case study using a recently discovered fire salamander (*Salamandra salamandra*) population near the index outbreak site, free of *Batrachochytrium salamandrivorans*

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Disease prevalence in fragmented populations is usually higher than in undisturbed areas because these populations suffer from demographic and genetic stochastic processes that lead to reduced fitness (Pearman and Garner 2005; Luquet et al 2012; Jousimo et al 2014) or alternatively because the contact rate of infected and non-infected individuals is higher due to the absence of predators and secondary hosts that otherwise decrease pathogen pressure (Allan et al, 2003; Greer & Collins 2008; Rudnick et al 2012). Increasing landscape permeability to facilitate gene transfer between neighboring populations is often applied as conservation technique. However, it also affects the rate and pattern of disease spread (McCallum, 2008; Rudnick et al 2012; Gottdenker et al 2014). Here, we describe the discovery of a new population of fire salamanders close to the index outbreak site of the chytrid fungus *Batrachochytrium salamandrivorans* and by using 18 microsatellite loci (Caspers et al, 2009), we show that the two fire salamander populations can be considered as one population on a regional scale. Next, we discuss the natural spread of the fungus (Stegen et al. 2017) and show that it is a very poor natural spreader, supported by field data from skin swabs tested for *B. salamandrivorans* and an in vivo experiment showing the lack of disease transmission between physically separated individuals suggesting that a physical barrier might be a suitable management tool to prevent the natural spread of the disease emphasizing the importance of

proper field hygiene measures.

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MICRO CT-WORKSHOP

Micro-computed tomography (micro-CT) in herpetological research: concepts, practical considerations and potential

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Micro-computed tomography (micro-CT) is a fast growing method in scientific research applications that allows for non-destructive imaging of morphological structures (HOLDSWORTH & THORNTON 2002). The method confers a strong advantage over physical specimens, for example, because measurements are not limited to the external anatomy and can be obtained at high precision (BROECKHOVEN et al. 2016). Despite its numerous applications and capabilities, the use of micro-CT has not reached its full potential as researchers in the biological sciences are often unfamiliar with the technique and its process, which includes sample preparation, the scanning process itself and 3D reconstruction (DU PLESSIS et al. 2017). Lack of knowledge can result in poor scan quality and/or inability to extract adequate information for the required research question.

The purpose of the workshop is two-fold: (i) to describe the various steps required for successful planning of research projects that involve micro-CT and make new users familiar with the relevant set-up, scanning, reconstructing and visualization methods, as well as terminology; (ii) demonstrate the potential that micro-CT offers for herpetology by addressing topics such as data extraction, measurement advantages over traditional methods and *in vivo* micro-CT scanning. Throughout the workshop, herpetofauna will be used as interactive examples. Ultimately, our aim is to improve the efficiency of herpetological research through an improved understanding of the capabilities and limitations of micro-CT scanning.

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Sexual dimorphism in osteoderm expression and the role of male intrasexual aggression

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The functional significance of osteoderms, body plates embedded in the skin of various extinct and extant tetrapods, has been studied widely in the past (reviewed in VICKARYOUS & SIRE 2009), leading to the advancement of a plethora of hypotheses that explain its presence (SEIDEL 1979, DACKE et al. 2015, VICKARYOUS et al. 2015). Whereas the emphasis of most studies is on the role of osteoderms as protection against predators (BROECKHOVEN et al. 2015), alternative hypotheses remain largely unexplored. This study investigates whether male intrasexual aggression might play contribute to variation in osteoderm expression using the Cape cliff lizard *Hemicordylus capensis* as model organism. Micro-computed tomography was used to examine ontogenetic variation and sexual dimorphism in osteoderm expression in two study populations that were assumed to display contrasting levels of male aggression. Our results show that osteoderms in the trunk develop at the onset or after sexual maturity in a lateral to medial fashion. A clear difference in osteoderm volume between males and females is detected, with osteoderm volume being significantly higher in males than in females, regardless of locality. Higher levels of intrasexual aggression, inferred from bite force data, appear to be present in individuals from the southern locality, and this appears to coincide with high osteoderm expression. In summary, although osteoderm expression has long been regarded a consequence of natural selection, our results suggest that these structures might instead be sexually-selected.

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Diversity of South American wormsnakes of the genus *Epictia*: Analysis of internal morphological characters via Micro-CT images

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The blind snake family Leptotyphlopidae STEJNEGER, 1892 consists of about 139 fossorial species with vestigial eyes that spend most of their time buried in loose soil, or under stones or logs, making their detection in the field difficult. Due to their small size, low number in diagnostic characters, rare observations on wild individuals, rarity in museum collections, and frequent lack of information of live colorations, the knowledge on intraspecific variation is insufficient in many species, making it a very complicated group to study. Among all snakes, the Leptotyphlopidae are unsurpassed in difficulty of identification, counting scales, determining head shields, accurately illustrating, and discovering diagnostic characters of systematic and taxonomic value. The neotropical genus *Epictia* Gray, 1845 is currently the most species richest group in the family.

Taxonomic review of the genus and studies carried out in unexplored regions resulted in recent descriptions of 11 new species in only the last 2 years, augmenting the number to about 43 known species in the genus. Nevertheless, due to the problems mentioned above discrepancies still exist on the exact number of currently recognized species within this group. Without knowing the exact number of taxa and the distributional range of each species, the assessment of the conservation status is also hindered.

The aim of the present study was to fill knowledge gaps and facilitate future research in the genus *Epictia*. Additionally, micro-CT images of ZFMK and SMF specimens were generated to uncover differences in the skull morphology. Gathered information were complemented with available data from the literature to detect intraspecific variation and find characteristic differences between species.

Flesh and bone: an integrative approach towards sexual size dimorphism of spectacled salamanders

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Males and females face different selection pressures due to a biased investment into reproduction. This does often result in different morphologies of the sexes. Sexual size dimorphisms (SSD) can give us important hints on the evolution and biology of a species. Salamanders are a well suited system investigating SSD including a diversity of reproductive modes and behaviours and patterns of SSD combined with life history traits including phylogeny help us to understand the evolution behind these processes. Because phylogenetically spectacled salamanders (genus *Salamandrina*) are the most basal taxon of the Salamandridae they have a key role for reconstructing the evolutionary pattern of SSD. Extensive morphological measurements on specimens of *Salamandrina perspicillata* gave us an overall overview of the expressed SSD in the external morphology but we employed high resolution micro CT scans of the skeleton to access SSD in skull, limbs and the pelvic griddle. 28 out of 43 characters investigated showed a significant dimorphism whereas males generally had larger limbs, heads and cloaca measurements relative to snout-vent length, while females showed larger trunks. Furthermore, the osteological analysis pointed out dimorphism, variation and anomalies in bone-counts. The novel dimorphic characters in the external morphology and osteology are likely linked to the different reproductive roles of the sexes of the salamanders.

Micro-CT, morphometrics, and phylotranscriptomics shed light on the underpinnings of convergent miniaturization in a radiation of frogs

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Madagascar's most diverse radiation of microhylid frogs, the subfamily Cophylinae with over 140 species and candidate species, are a potential model system for the study of convergent miniaturization of body size. The species encompass a wide range of ecologies from fossorial to arboreal, and an equally wide range of body sizes, from diminutive species below 9 mm in adult body length, to large heavy-bodied frogs of over 100 mm. Based on a multi-gene phylogeny with almost complete taxon sampling, we can show that neither size nor ecology are strongly phylogenetically assortative across the subfamily, but have instead converged and diverged repeatedly over their diversification. The extent of convergence is such that signals of phylogeny from morphometry or skeletal anatomy are obscured. However, correlates of miniaturization emerge clearly from micro-CT data with a nearly complete taxon sampling. Miniaturization has occurred through paedomorphism in most taxa, but at least partially through proportional dwarfism in one exception.

MEETING

***REGIONAL IUCN VIPER SPECIALIST TAXONOMY
WORKING GROUP FOR EUROPE AND NORTH ASIA***

Regional IUCN Viper Specialist Taxonomy Working Group for Europe and North Asia

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The Regional IUCN Viper Specialist Taxonomy Working Group for Europe and North Asia was established at the 5th Biology of Vipers conference in Morocco in May 2017, with the purpose to facilitate updating of the regional viper species list, which is a necessary prerequisite for reassessment of the Red List status of regional viper species and recognition of priorities for conservation actions in the next three years. According to recent publications, new species should be included in the IUCN Red List. Moreover, due to new data about distribution, phylogeny, etc., the status of some viper species that already occur on the list Red List must be promptly re-assessed.

All interested participants of 19th SEH congress are encouraged to take part in the European VSG Taxonomy Working Group meeting which will start on September 19th 2017 at 13:45h, be managed by the European VSG coordinator and hosted by the Organizing Committee of the 19th SEH congress and the University of Salzburg.

ORAL PRESENTATIONS

Damaged by time and formalin: Using ancient DNA technology to recover century-old snake mitogenomes

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The Natural History Museum of Denmark is housing a remarkable herpetological collection with many thousands of specimens collected across centuries. A large proportion of the animals have, however, been formalin-fixed during preservation which is known to seriously compromise the potential for molecular research.

Using carefully optimized protocols normally applied to ancient DNA research (ALLENTOFT et al. 2015), combined with Next Generation Sequencing, we have recently received highly encouraging results from a number of such specimens. We present the methodology and showcase genetic results from the danish population of Smooth Snake (*Coronella austriaca*) that went locally extinct in the early 20th century.

Despite displaying severely degraded DNA (av. fragment length <50bp, C-T damage rates >10%), we managed to assemble the complete mitochondrial genomes of three danish *C. austriaca* individuals, and combined these data with mitogenomes of >30 modern individuals from across Europe, sequenced specifically for this study. Phylogenetic and network-based analyses showed that the danish population had very high genetic similarity with the current Scandinavian populations (posterior clade probability = 1) and was thus not related with the *C. austriaca* lineages present today in Central-Western Europe. We discuss the phylogeographic implications of these findings. More broadly, we discuss the potential for analysing highly degraded DNA (whether damaged by time or formalin or both) obtained from herpetological tissues.

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To the problem of caudal autotomy and regeneration in Asian mountain agamas

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We studied the cases of caudal autotomy and regeneration in agamid lizards of the *Paralaudakia* genus. Agamids in general including Asian rock agamas lack autotomy plans in the caudal vertebrae.

We examined 185 specimens presented by series of *P. caucasia* (115 specimens), *P. erythrogaster* (9 specimens), *P. lehmanni* (27 specimens) and *P. stoliczkana* (31 specimens) in the collections of Zoological Institute, Russian Academy of Science, St.Petersburg using the X-ray examination to determine the type of autotomy.

We found that intervertebral tail breaks and following regeneration in agamids are not so rare (32% and 25% correspondingly) as it was supposed earlier (ARNOLD, 1984). The case of bifurcation of regenerated tail was registered in *P.caucasia*. In agamids the blastems following intervebral regeneration is formed at the rupture site in the area of the intervertebral disc. The clubbed regenerated tails in agamids (SHALL et al., 1988), the frequencies of autotomy and regeneration in different species of Asian mountain agamas of *Paralaudakia* genus and potential use of regenerated tails in social interactions and escape strategies are discussed.

The work was supported by the Grant of the Russian Foundation of Basic Research (RFBR 15-04-01730).

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A new species of dragon lizard from Swaziland and South Africa

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The dragon lizards of southern Africa were placed in a separate genus, *Smaug*, following a molecular phylogeny of the family by STANLEY et al. (2011). Subsequently, a multi-gene molecular phylogeny of the genus *Smaug*, conducted by STANLEY & BATES (2014), identified a south-eastern clade (Swaziland and adjacent areas in South Africa) of relatively lightly armoured, geographically proximate species (*S. warreni* and *S. barbertonensis*). While this relationship was not unexpected, the analysis also determined that some specimens from Swaziland, identified as *S. barbertonensis*, were in fact most closely related to *S. warreni* (a species restricted to the Lebombo Mountains) and represented a distinct ‘cryptic’ species. Examination of voucher specimens used in the analysis, as well as most other available museum material, indicated that the new species is readily distinguishable from *S. barbertonensis* (and *S. warreni*) by its unique dorsal, lateral and ventral colour patterns, together with a few differences in scalation features. The new species occurs in Swaziland and small areas in adjacent South Africa. Recognition of the new taxon means that the range of *S. barbertonensis* is now much restricted (Barberton-Nelspruit area) and will require a new conservation threat assessment.

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Discovering Hidden Diversity in the *Hemidactylus* (Reptilia: Gekkonidae) of Africa

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Hemidactylus is the second-most species-rich genus of geckos and the only one to have radiated widely in Africa, tropical Asia, and the Palearctic. Although relationships among *Hemidactylus* of some regions have been well studied, only a few groups in sub-Saharan Africa have been investigated. We used a multilocus molecular phylogenetic approach to identify genetic diversity within four main groups of African *Hemidactylus* (*H. mabouia* complex, *H. angulatus* complex, Horn of Africa endemics, West and Central African clade) and as a first step to delimit species boundaries in preparation for a thorough taxonomic revision and zoogeographic assessment of the genus in Africa. Both *H. mabouia* and *H. angulatus* comprise a large number of geographically circumscribed species that share a similar morphology, but cannot be characterized as truly cryptic. In both cases diversity is lower in West and Central Africa than in other areas of the respective ranges of these complexes. It appears that invasive populations of *H. mabouia* in the New World all represent the Central African taxon and that southeastern Africa supports hitherto unexpected diversity in this clade. Several deeply divergent species in the *H. angulatus* complex are present in Ethiopia, complementing an already rich fauna of endemics with affinities to Arabia. A clade of West and Central African *Hemidactylus* (exclusive of the *H. fasciatus* group) extends from southern Angola to the Western Guinean forest block. Allocation of names to lineages in this clade has historically been difficult and our results suggest extensive localized endemism, particularly from Angola to Cameroon.

Genetic diversity of *Pelophylax* species in south-western Balkans

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Water frogs of the genus *Pelophylax* comprise 12 species and three asexual hybrid taxa in the western Palearctic. The continental part of the Balkan Peninsula is inhabited by five species, whose distribution is poorly known. Therefore, we decided to shed more light to diversity of these relatively common and widely distributed, but still overlooked amphibians in the Balkans.

During years 2014 – 2017 we collected a total of 219 *Pelophylax* individuals from 85 localities across western and south-western Balkan Peninsula. We analyzed sequences of the mitochondrial ND2 gene and nuclear fragment for serum albumin intron-1 (SAI).

Based on nuclear data the most of analyzed specimens were recognized as *P. ridibundus*. This species was found in Albania, Bosnia and Herzegovina, Croatia, Greece, FYROM, Montenegro and Serbia. Rest of specimens were assigned into *P. epeiroticus*, *P. bedriagae* and *P. shqipericus*. While *P. epeiroticus* was found in Greece and southern Albania, *P. bedriagae* was collected from Bosnia and Herzegovina only. Two individuals assigned to *P. shqipericus* were collected from southern Montenegro and north-western Albania. Analysis of mitochondrial diversity recognized 73 haplotypes (Hd: 0.931), while the majority of them were assigned to *P. ridibundus* (63), eight and two haplotypes were recognized in *P. epeiroticus* and *P. shqipericus*, respectively. The largest haplotype diversity was observed in southern and south-eastern Albania. We also observed occasional discrepancies between nuclear and mitochondrial signal, which could be attributed either to interspecies introgression of mitochondrial DNA or incomplete lineage sorting.

Explanation for Reproductive Abnormalities in the European Blind Cave Salamander, *Proteus anguinus*?

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Our current research shows that the vulnerable and endangered cave salamander, *Proteus anguinus*, is characterized by a range of extraordinary demographic and reproductive peculiarities. These abnormalities include a skewed sex ratio (approximately 2:1) in favor of females, as well as gonad abnormalities, including a high percentage of atretic follicles in the ovaries, male specimens with testis-ova in their testes, and a recently discovered hermaphroditic specimen. Similar gonad abnormalities in other species of amphibians are usually associated with hormonal dysfunction (Ogielska, 2009) or exposure to environmental endocrine disruptors (Hayes et al, 2011, Lambret et al 2015).

In *Proteus*, we think that the observed reproductive peculiarities, especially the testis-ova and hermaphroditism, are explained by a translocation between the X and Y sex chromosomes (Sessions et al. 2016) disrupt the function of genes involved in sex determination. Our current research is focused on testing the hypothesis that this translocation has a positive selective advantage because it is involved in the evolution of genetic mechanisms of cave adaptations in *Proteus*.

* The gonad studies were performed on preserved archived specimens from the permanent collection of the Department of Biology, UNI Ljubljana that had been collected from 1972 to 2009 for research purposes with permission of the Ministry of the Environment and Spatial Planning of the Republic of Slovenia (35701-81/2004-9 and 35601-1/2010-6).

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Host Niche May Determine Disease-Driven Extinction Risk

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The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) drives declines and extinctions in amphibian communities (Lips et al. 2006, Vredenburg et al. 2010, Cheng et al. 2011). However, not all regions and species are equally affected (Berger et al. 1998, Lips, Reeve & Witters 2003). To explore this concept in the montane cloud forest of Cusuco National Park, Honduras, environmental parameters, water filters, amphibian skin swabs and water samples used to assess the microeukaryote diversity and abundance were collected from bromeliad phytotelmata and streams, and their corresponding amphibians. Here, we show that association with amphibian aquatic habitat types (bromeliad phytotelmata versus stream) across Central America results in the odds of being threatened by *Bd* being five times higher in stream microhabitats. This differential threat of *Bd* was corroborated by a significantly lower prevalence of *Bd* in bromeliad-associated amphibian species (3.4%) compared to riparian species (12.4%) in Honduran cloud forests (Fisher's exact test, $p = 0.008$). The lower conduciveness of the bromeliad environment for *Bd* transmission is exemplified by significantly less suitable physicochemical conditions (Temperature: $W = 687.5$, $p < 0.001$, PH: $W = 0$, $p < 0.001$) and significantly higher abundances of *Bd*-ingesting micro-eukaryotes present in bromeliad water ($W = 647$, $p < 0.001$), which preclude aquatic *Bd* zoospore survival and the development of an environmental reservoir of the pathogen. Bromeliad phytotelmata thus act as environmental refuges from *Bd*, which contribute to protecting associated amphibian communities against chytridiomycosis-driven amphibian declines that threaten the syntopic riparian communities.

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Chronic exposure to a glyphosate-based herbicide makes common toad (*Bufo bufo*) larvae more toxic

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Pesticides can lead to mortality, or have sub-lethal effects on non-target organisms, leading to complex fitness consequences. Many animals use defensive chemicals to protect themselves against predators and diseases, but the effects of contaminants on the production of these chemicals are poorly known. Amphibians are considered especially vulnerable to environmental contaminants due to their unshelled eggs, highly permeable skin, and complex life-cycle. At the same time many species synthesize or sequester defensive chemicals.

To investigate how exposure to a glyphosate-based herbicide affects the production of bufadienolides in common toads (*Bufo bufo*), we exposed tadpoles to three concentrations (0,2 and 4 mg a.e./ L) of the herbicide under laboratory and outdoor mesocosm conditions.

In both experiments, herbicide exposure increased the amount of bufadienolides in toad tadpoles. In the laboratory, individuals exposed to 4 mg a.e. / L glyphosate throughout their larval development had higher bufadienolide content at metamorphosis than non-exposed tadpoles, whereas exposure for 9 days to the same concentration or to 2 mg a.e. / L throughout larval development or for 9 days had no detectable effect. In outdoor mesocosms, tadpoles from 16 populations exhibited elevated bufadienolide content after a 3-weeks exposure to both concentrations of the herbicide.

According to our results, pesticides can affect non-target organisms and their chemical defenses, which in turn may affect predator-prey dynamics.

Isolated populations of *Salamandra atra* at the southern periphery of the species range: genetic variation and evolutionary differentiation

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Differently than most other European amphibians, *Salamandra atra* is fully terrestrial and cold-adapted. Its geographical range is constrained by narrow ecological tolerance and low dispersal ability. The species is broadly present in the northern part of the Alps, whereas only isolated populations are sparse across the southern Prealps and the Dinarides, including the relatively elusive and phenotypically differentiated *S. a. aurorae* and *S. a. pasubiensis*.

We aimed at assessing the genetic diversity and evolutionary divergence of the southern separate populations of *S. atra*. We revised old and recent evidence on the occurrence of the species along the southern Prealps and the Dinarides. Through intense field surveys from the Orobic mountains, along the Dolomites and Venetian Prealps, down to the Dinarides, we assembled an unprecedented sample for molecular analyses: more than 100 individuals from 14 sites.

By employing different markers (10 autosomal microsatellite loci, >2000 bp mitochondrial sequences, 2 protein-coding nuclear genes) and different analytical approaches (populations genetics, phylogeographic and phylogenetic analyses), we found that most populations surviving on the southern Prealps are genetically strongly isolated and differentiated, even when apparently not differing in skin coloration. Values of genetic diversity differ across populations, being consistently lower in comparison with populations in the northern part of the Alps. Moreover, we found evidence for a deep evolutionary separation between all southern prealpine and dinaric populations and those inhabiting the remaining part of the Alps.

The effect of thermal acclimation on metabolic rate, damage load and antioxidant capacity in the grass snake (*Natrix natrix*)

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Reactive oxygen species (ROS) are by-products of aerobic metabolism and cause damage to various biomolecules. Traditionally, a positive relationship between aerobic metabolism and damage is assumed, but recently a negative relationship has been reported in fish. Here we acclimated ectothermic grass snake (*NATRIX NATRIX*) to different ambient temperatures with the goal to investigate the effect of temperature manipulation on standard metabolic rate (SMR), damage (dROM and micronuclei count) and antioxidant capacity (Oxy-adsorbent). Snakes were exposed for six months to either cold (18°C) or a warm (32°C) ambient temperature that both lie within a range allowing the snakes' activity and that are known to impose changes in metabolic rates.

Temperature manipulation significantly affected SMR, with four-times higher SMR in warm-acclimated snakes and lower SMR in cold-acclimated individuals. Damage (dROM and micronuclei count) was significantly lower in warm-acclimated snakes. Antioxidant capacity did not differ between groups. Temperature and SMR are tightly coupled in ectotherms so the effect of treatment on damage and antioxidants is most likely mediated by SMR. Our results corroborate novel findings for a negative relationship between SMR and damage in fish. Lower amount of damage in warm-acclimated snakes might be explained by multiple and mutually non-exclusive effects of higher mitochondrial uncoupling, increased rates of damage repair or removal. Our study suggests a fundamental cost of living in colder compared to warmer environment: lower energy requirements for self-maintenance are set-off by higher amounts of damage to biological structures.

Finding evidence-based amphibian conservation strategies in an emergency

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In the face of global amphibian declines, evidence-based strategies for species conservation are increasingly advocated. In most cases, proper conservation strategies are difficult to identify because there is limited knowledge about the natural system and uncertainty about how our actions can affect it. In the face of urgent imminent threats, science should focus on the questions of most immediate management relevance.

In this study, we used an integral projection model to explore potential mitigation actions for avoiding population declines and the ongoing spatial spread of the fungus *Batrachochytrium salamandrivorans* (*Bsal*). Available evidence suggests that a *Bsal* outbreak in a fire salamander (*Salamandra salamandra*) population will lead to its rapid extirpation. Treatments such as antifungals or probiotics would need to almost entirely interrupt

transmission (reduce probability of infection by more than 95%) in order to reduce the risk of host extirpation and successfully eradicate the pathogen.

Improving the survival of infected hosts is most likely to be detrimental as it increases the potential for pathogen transmission and spread. Active removal of host species has some potential to reduce the spread of *Bsal*, but its effectiveness depends on the presence of *Bsal* reservoirs and on the host's spatial dynamics, which should therefore represent research priorities. Mitigation of *Bsal* epidemics in susceptible host species is highly unlikely, requiring effective interruption of transmission and substantial removal of host individuals. More generally, our study illustrates the advantages of framing scientific analysis directly in the context of management questions, rather than adapting to it *a posteriori*.

Nomenclature and taxonomic status of the genus *Blanus* Wagler, 1830 (Squamata: Amphisbaenidae) from the Iberian Peninsula

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Presently two species of the genus *Blanus* are known from the Iberian Peninsula: *Blanus cinereus* (Vandelli, 1797) and *Blanus mariae* Albert & Fernández, 2009. The latter was recently described based on molecular studies which pointed to the existence of two well separated lineages in the peninsula. However the description of *B. mariae* presented several flaws that render the description invalid, namely the designation of an invalid lectotype, the application of *B. cinereus* to a different lineage than the one for which it was originally described, and the apparent disregard for the potentially applicable available nomina *Amphisbaena* [= *Blanus*] *rufa* Hemprich 1820 and *Amphisbaena* [= *Blanus*] *oxyura* Wagler, 1824. Based on both historical and molecular data, we present evidence that 1) allows the proper identification of the original *B. cinereus* type locality and consequent lineage identification; 2) elucidates the origin and invalidity of the purposed lectotype; and 3) proposes a new nomenclature for the Iberian *Blanus*. *Blanus mariae* is considered a junior synonym of *B. cinereus* and *Blanus rufa* is a candidate name that may apply to the central Iberian clade of *Blanus*. We discuss the main nomenclatural, taxonomic and conservation implications of this change, as also discuss the importance of solid historical, nomenclatural and taxonomic data when describing new species or proposing new nomenclatural acts.

The effects of invasion history on the intraspecific competitive ability of cane toads (*Rhinella marina*) in Australia?

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Since their introduction to Australia in 1935, the invasive cane toad (*Rhinella marina*) has rapidly colonised northern Australia, causing vast ecological disturbances. Over their expansive Australian range, cane toads have evolved high dispersal phenotypes increasing their rate of colonisation. However, these adaptations have come at the cost of other fitness traits, resulting in poorer overall survival. How, then, has the rapid evolution of phenotypes across their range affected competition strategies?

To explore the effects of invasion history on intraspecific competitive ability, we conducted trials that placed the offspring from invasion range edge and range core populations -representing an 80-year difference in time since colonisation- in direct competition for resources. Our results show that larval-stage offspring from established populations outcompeted those from the invasion front, with higher levels of growth and successful metamorphosis. However, in the early terrestrial stages, the opposite trend is shown, with invasion front individuals outperforming core individuals both in foraging trials and growth. How, then, does this translate to a real-world situation with flow-on effects between each life stage? The answer to this question is vital for the proposed introduction of the core population genotype ahead of the evolved invasion front colonisers, which aims to slow the rate of colonisation. Current models predict the success of this strategy, however further data from small-scale field simulations of population mixing will greatly increase their accuracy.

Out in the cold: adaptive strategies of the Common Frog (*Rana temporaria*) in extreme habitats

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We studied the mechanisms that allow the Common Frog to persist in extreme environments, in an attempt to understand how global changes might affect its distribution. We compared (i) size and age-related parameters using skeletochronology over a 12 years period, and (ii) feeding strategies using stomach flush and estimates of trophic resource availability in Common frog populations. We studied three populations inhabiting subarctic (690N, 600 m a.s.l.) and alpine (450N, 2050 m a.s.l.) habitats, and compared with a low altitude (450N, 900 m a.s.l.) valley population, as control.

We found a significant variation in size and age-related parameters in the Common Frog, including age at sexual maturity, mean age and longevity (i.e. maximum age class), between sexes and populations inhabiting contrasting environments. Also we observed variations in age related parameters over short spatial and temporal scales.

Common frogs are generalist predators, foraging on their main food item and some secondary preys in similar proportion to their environmental availability. The differences in prey abundance and availability among sites were also reflected in the food consumed. We observed a certain degree of selectivity in feeding, with preference for some taxa or size categories. Adults consumed more prey individuals and had a higher prey taxa diversity than juveniles. Juvenile feeding varied among sites, with more prey individuals and higher prey taxa diversity in the high latitude population. Overall, small-scale spatial and temporal variation in environmental conditions can induce significant changes in important life-history traits In the Common Frog.

Micronuclei include genome eliminated during gametogenesis of di- and triploid interspecies hybrids from *Pelophylax esculentus* complex

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To reproduce some interspecies hybrids employ elimination of one parental genome and endoreplication of the remaining one during gametogenesis. However, cytological mechanisms of these processes remain unknown. Genome elimination and endoreplication were found in interspecies hybrids from European water frog complex (*Pelophylax esculentus* complex). The complex includes two parental species: *P. ridibundus* (RR genotype), *P. lessonae* (LL genotype), and their hybridogenetic diploid (RL) and triploid (LLR, RRL) forms of *P. esculentus*.

To detect genome elimination during hybrids gametogenesis we performed artificial crossing experiments followed by identification of tadpoles karyotypes. The results suggest that diploid hybrid males and some females usually eliminate the L genome while triploid individuals with LLR genotype eliminate the R genome. In gonads dissected from hybrids, but not parental tadpoles, we revealed micronuclei in cytoplasm of germ cells. To identify genome included in micronuclei we performed FISH with probe specific to *P. ridibundus* centromeric sequences. In each gonad of 8 triploid tadpoles with LLR genotype we found R chromosomes in 80% of observed micronuclei. While in each gonad of 15 diploid hybrid tadpoles we found R chromosomes only in 10% of observed micronuclei. Thus about 90% of micronuclei included either acentric R chromosome fragments or L chromosomes. We conclude that genome is subsequently eliminated via micronuclei formation.

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Allopatric divergence in a North African Palearctic relict: the role of environmental factors and niche conservatism in shaping the genetic structure of *Salamandra algira*

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The complex topography of North Africa, together with the climatic oscillations of the Pleistocene, have contributed to a remarkable intra-species diversification in the region, promoting isolation, divergence, and potential adaptation to local environmental conditions (Husemann et al., 2014). The evolutionary history of *Salamandra algira*, a Palearctic relict in northern Maghreb (e.g. Ben Hassine et al., 2016; Merabet et al., 2016), was likely influenced by past climatic oscillations, resulting in four allopatric lineages (subspecies) presently restricted to some mountainous ranges of Morocco and Algeria (Beukema et al., 2010, 2013). We assess the spatial genetic structure of *S. algira* across its entire distribution (82 localities) using mtDNA and microsatellites in order to delineate the boundaries of all lineages. Based on the uncovered population structure, we perform ecological niche-based models and niche overlap tests to investigate the effect of environmental variability on the diversification observed within *S. algira*, as well as to identify potential paleoclimatic refugia for each lineage during the Pleistocene. Lineages showed distinct responses to climatic and habitat factors, and independent areas of climatic stability were identified for each major lineage. Nonetheless, ecological niches were found to be predominantly conserved across *S. algira* lineages, suggesting differences in environmental affinities are predominantly a result of differences in habitat availability. These results point to allopatric divergence within Paleoclimatic refugia as the most likely model for the diversification of *S. algira*.

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Temporal migration patterns and mating tactics influence size-assortative mating in *Rana temporaria*

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In sexually reproducing species pairs are more likely to be formed if phenotypic traits are similar between male and female. These assortative mating can be found in a wide variety of taxa, but the behavioral mechanisms behind are rarely examined.

We aimed to understand the underlying mechanisms that lead to size-assortative mating in a widely distributed, explosive breeding amphibian species, the European common frog (*Rana temporaria*). Therefore, we use data from pairs at different breeding sites collected over several years and show a consistent pattern of size-assortative mating in natural populations. We asked, if size-assortment is a result of temporal migration patterns of differently sized individuals. Additionally, we conducted experiments to test for adaptive mechanisms like mate competition between males and fertilization success of different size-matched pairs. We observed a migration pattern where larger individuals arrived first at the breeding sites, therefore leading to differences in mate availability and supporting size-assortment during migration.

We detected a random mate choice behavior of small males, which could be due to large male advantage in scramble competition in the breeding aggregation. By being less picky in choosing a mate, small males could minimize male competition and the chance to get access to a female will increase. The size-assortment of pairs does not influence fertilization success, but female fecundity correlates with body size and by choosing a large female, males could maximize the number of their offspring. We show that mate finding in anurans is a multicausal process and more complex than formerly expected.

Chameleons, from the genus *Bradypodion*, are what they eat... with some gender inequalities

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Chameleons show unique anatomical characteristics of their feeding apparatus (i.e. projectile tongue), along with a feeding strategy that does not fall into the classical feeding strategies, as chameleons are considered cruise foragers, an intermediate strategy between sit-and-wait and active foragers (Butler 2005; Measey, Raselimanana & Herrel 2014). Therefore, the relationships between bite force, head shape, and diet may differ from those reported for other terrestrial organisms.

Here, we investigated the relationships between head shape, bite performance and diet in 14 of the 17 extant *Bradypodion* species to determine whether variation in diet can explain the observed diversity in bite force and head shape in this genus. We also evaluate differences between sexes in terms of the relationships between head shape, bite force and diet and predict tighter relationships in females given that the head in this sex is principally under natural selection.

Our results strongly support the hypothesis for selection for head shapes adapted for eating large or hard prey, but also suggest a role of sexual selection in driving the evolution of bite force and head shape.

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Species distribution modeling and field survey on *Iberolacerta horvathi* (MÉHELY, 1904) in Austria: predicted and actual habitats considering topographical, geological and bioclimatic parameters

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For the ongoing research project we developed and applied the first species distribution model (SDM) on *Iberolacerta horvathi* (MÉHELY, 1904) for the Austrian territory. The known distribution pattern in southern Austria is highly fragmented. Since its first discovery for Austria in 1986, approximately 600 individuals at 50 sites have been recorded, which suggests that the range of *I. horvathi* here, at the northernmost boundary of its natural populations, might still be underestimated.

The primary aim of this study is to detect new populations and its potential habitats. For the correlative modeling-approach we analyzed and implemented suitable habitat parameters using ArcGIS and Maxent based on 48 occurrence records. To represent the biotic and abiotic conditions in these localities we considered geological, topographical, bioclimatic and land cover information obtained by 16 different GIS layers. In addition to the included statistical performance tests, the prediction performance will be tested in the field and the obtained results will improve our understanding on species ecological preferences on the territory of Austria.

Spatial planning for herpetofauna conservation: developing and using metrics for setting goals and taking forward action

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The conservation status of a species is defined by biological parameters which, in turn, are impacted by a range of policy and practical conservation measures, pressures and threats. The main focus for conservation status assessment to date is reporting, e.g. through the Habitats Directive. However, effective status assessments, using measurable parameters (metrics) and with defined measurable goals, allows an objective programme of impact assessment and positive conservation action to be developed.

Great crested newts *Triturus cristatus* are widely distributed in the UK; there it has been the focus of considerable attention, most significantly through concerns that the species' protected status impacts adversely on commercial interests.

Recent work has seen the stepwise development of status assessments at different scales, methods for quantifying impacts, notably through construction, and objective approaches for determining favourable levels for different status parameters. These features can also be mapped, providing a spatial plan that allows targeted conservation efforts.

Improvements in technology and surveillance techniques, notably species distribution modelling, GIS, digitised environmental data and environmental DNA sampling have meant that a range of key metrics can be identified and quantified where previously this would not have been possible. With further technological advances, e.g. remote sensing, greater use of spatial planning for species is anticipated.

The presentation draws together elements from a number of different initiatives to propose a common framework for taking forward conservation activities and for developing surveillance. While the focus is on great crested newts, the application to other UK herpetofauna is discussed.

Distribution and ecology of the Fire Salamander in Tyrol - notes from the regional range boundary within the Alps

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In 2016 and 2017, intensive field surveys were used to study the distribution, habitat requirements and potential conservation problems of *Salamandra salamandra* at its western distribution boundary in Tyrol, Austria. Additionally a public platform to report salamander sightings was set up.

Compared to data from lowland habitats, larval densities in Tyrol are low. In 32 (45.1%) of 67 investigated water bodies a total of 310 larvae were recorded. Although artificial reservoirs showed the highest larval densities, (semi)natural springs and streamlets were quantitatively the most important reproduction sites. Larval densities were positively correlated with the number of pools, coverage of gravel, mud and dead leaves, shadow, the structural quality of the aquatic habitat and the amount of woodland within a 100 m buffer zone. High water temperature, increased drift risk and the percentage of agricultural land within the buffer zone had a negative impact on larval densities.

Visual encounter searches for adult salamanders usually yielded only small numbers of adults (average one individual / hour), signalling small populations. Analysis of actual versus potential woodland composition at salamander sites showed a clear preference for beech forests, but also a high acceptance for primary and secondary coniferous forest.

The majority of reported sightings came from well known populations in the eastern part of Tyrol. However, a small number of reliable reports suggest the occurrence of small, isolated populations west of the original distribution boundary.

The results from the field surveys as well as the data gathered on the reporting platform indicate generally small population sizes in Tyrol. Currently, habitat destruction poses the greatest threat to *Salamandra salamandra* in this area.

Antipredator response and agility in terrestrial and aquatic Chelonians – steady as a rock or running back to water

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The shape of Chelonian's shell, i.e. the length of bony bridges between plastron and carapace, physically limits movements and thus affects agility (Bonnet et al. 2001). Besides these function-morphological restrictions, behavioural characteristics may additionally influence mobility. To test this hypothesis, I compared speed and efficiency of European pond turtles (*Emys orbicularis*) and Hermann's tortoises (*Testudo hermanni*) in two field experiments: (1) self righting when overturned on the back and (2) releasing from a rope stuck on front part of their plastron, which mimics coping with vegetation. Both tests represent situations with which these animals deal in everyday movements. European pond turtles were faster and more efficient in both tests. They primarily used their long necks as a lever to flip back on legs, while Hermann's tortoises relied on vigorous legs movements (Domokos & Varkonyi 2008).

Additionally, European pond turtles were bolder to start moving shortly after handling. These findings are in accordance with antipredator tactics observed in these two species. When close to water, *E. orbicularis* promptly dive in at first sign of potential aerial or terrestrial predator (Martín et al. 2005). On land, further from water body, *E. orbicularis* keeps the swiftness of reaction and does not rely on hiding inside the shell as much as *T. hermanni*. Adult European pond turtles are more prone to terrestrial predators such as dogs and boars, due to flat shell and narrow bridges, compared to adult tortoises with more domed and enclosed shells (Dosik & Stayton 2016).

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Morphological trade-offs between duration and maximal capacity of bite performance in *Podarcis bocagei*

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Biting performance in lizards is important for mating, feeding, and territory defense. One of the most studied functional traits is maximal bite force, but other aspects of biting are rarely considered. Here we examined maximal biting capacity and bite duration in *Podarcis bocagei*, under different conditions designed to simulate feeding and antagonistic behavior to. We 1) investigate sexual dimorphism, 2) examine how performance varies across ecological and social tasks, 3) test for trade-offs between maximal capacity and duration, and 4) explore the relationship between head morphology and bite performance. Our results show that males bite harder than females, but also that they sustain their bites for longer which may have important implications for mating, prey capture and handling. We also found that males, but not females, exhibited higher bite forces in tests simulating feeding as compared to those simulating antagonistic behavior. We found no trade-off between maximal biting capacity and duration when examining raw performance data, but a marked trade-off existed between these performance parameters when head morphology was taken into account. Lizards of both sexes with longer and wider heads bit harder but for shorter intervals. This pattern was also observed among males alone, but not when examining only females. Put together, our findings demonstrate the importance of bite performance in ecological tasks, and in social behavior in males. Remarkably, they also suggest the existence of trade-offs in the morphological optimization of maximal capacity and bite duration, possibly due to physiological constraints on muscle composition, insertion, and orientation.

A window to the heart development of Squamata: cardiac conduction system and level of ventricular septation

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Birds and mammals have a full ventricular septum and well developed cardiac conduction system. To study the association between the ventricular septum and conduction system, we focused on Squamata models, where the ventricular septum is almost full (monitors) or poorly developed (lizards typically).

Optically mapping of the electrical impulse over the ventricle and cardiac histology was evaluated in embryonic hearts of the central bearded dragon (*Pogona vitticeps*) and the spiny-tailed monitor (*Varanus acanthurus*). Subsequently, a series of mangrove monitor (*Varanus indicus*) embryonic hearts were evaluated using cardiac conduction markers (transcription factors *Irx1*, *Irx2*, *Tbx3*, and *Tbx5*), which are responsible for patterning of the vertebrate heart, and staining with HNK1 antibody, which detects a common neuroectodermal antigen expressed in peripheral nerves as well as developing cardiac conduction system. In *P. vitticeps*, optical mapping revealed primitive activation patterns in base-to-apex direction at both early and late stages. In the case of *V. acanthurus* the first ventricular epicardial breakthroughs were deep in the ventricle in the area of the forming interventricular septum on the left side of the late stage *V. acanthurus* ventricle. In *P. vitticeps*, HNK1 antibody labelled the mesenchymal structures, cardiac nerves, and also neural crest cells. In *V. indicus*, the HNK1 labelled mesenchymal structures, cardiac nerves, and a ring-like myocardial structure in the ventricular septum. Expression of *Irx1* and *Irx2*, *Tbx3* and *Tbx5* was detected in the partial ventricular septum in the *V. acanthurus* heart. It appears that a primordial conduction system is present also in some squamate reptiles.

Survival, area use and thermoregulation of reintroduced Hungarian meadow vipers (*Vipera ursinii rakosiensis*)

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The Hungarian meadow viper conservation program started to reintroduce captive bred vipers in 2010. Since then, 464 vipers were released to six habitats in Kiskunság and Fertő-Hanság National Parks in Hungary. During regular monitoring, we observed vipers 376 times, of which 236 times we managed to identify them, representing 127 individuals, 40 of them newly registered, originating from the 26 observed gravid females. The longest period between release and last observation was 1575 days. On average 15% of the released vipers were sighted at least once again.

In order to apply a remote tracking method, pre-programmed radio-tags with a detection range of 100-200 m were surgically implanted into the abdomen of selected vipers. Generally five snakes were tagged within each released group, resulting 33 tagged snakes. Each snake was localized at least once a week. Snakes had an average Minimum Convex Polygon of 0.43 ha (max. 1.51 ha), with an average distance between locations of 324 m (longest 921 m). Only eight snakes (24%) managed to overwinter. In 20 cases (60%) the snakes were killed by predation, half of them by avian predators like Common buzzards or Harriers and the other half by mammals, most likely red fox and badger. The most sensible periods to predation were the week after release and the early spring and late autumn periods.

The implants also operated as temperature loggers, recording data every five minutes. We recovered temperature logs of 16 snakes. Four of them managed to overwinter in natural habitat, spending on average 131 days hibernated at a mean temperature of 5.3 °C.

A global database of anomalies in natural populations of amphibians – and global patterns derived from the database

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Anomalies in animals have interested humans for centuries. The rediscovery of mass anomalies in natural populations of frogs in North America have sparked global concerns about the state of the environment and human health risks. The absence of a global database on anomalies in natural populations of amphibian hampers a comparison with other cases and the evaluation of new discoveries. Since the 1980ies I have started to develop and continuously update such a global database. The structure and content of the database is presented and emerging patterns are analysed. Data were extracted from more than 1770 publications and cover more than 3500 populations from 98 countries. The earliest publication dates back to 1554 and the number of publications has sharply risen in recent decades. Most publications report 1-2 abnormal individuals and 1-2 different types of anomalies. Whereas there has been no increase in the median percentage of abnormal individuals, the 75% upper quantile has risen sharply. In the USA the prevalence is higher outside than inside National Wildlife Refuges and National Parks. Differences in the patterns of the relative frequency with which different types of anomalies have been reported will be illustrated for anurans and urodeles, Among others, ectromely and anophthalmia has been reported for significantly fewer percentage of surveyed urodelean populations compared to anurans.

Structure and ultrastructure of the developing egg tooth in squamate reptiles

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The egg tooth plays major role in hatching of squamate reptiles enabling breakage of hard eggshells. This transient structure is present in most oviparous species of snakes and lizards. The general structure of the egg tooth is similar to the structure of typical tooth characteristic in all vertebrates. The egg tooth however, due to its function, exhibits structural differences in relation to regular teeth. That differences may also be reflected in the ultrastructure of the cells involved in its differentiation. Knowledge regarding the development of the egg tooth in reptiles is currently limited in comparison to the one on other vertebrate teeth. This issue is even more pronounced on cell biology level because there is no study devoted to submicroscopic differentiation of that structure.

Our study involves a detailed analysis of the egg tooth structure, mainly focusing on the ultrastructure of the cells engaged in egg tooth differentiation. We described the ultrastructure of differentiating enamel organ (inner enamel epithelium, stratum intermedium and outer enamel epithelium), dental pulp and odontoblasts in grass snake *Natrix natrix* and brown anole *Anolis sagrei* embryos, basing mostly on the light and transmission electron microscopy techniques.

Approvals for research were obtained from the Local Ethics Commission in Katowice (41/2010; 87/2015), the General Directorate for Environmental Protection of the Polish Ministry of Environment (ref. No: DOPozgiz-4200/II-88/4189/10/JRO) and the Regional Directorate for Environmental Protection in Katowice (WPN.6401.257.2015.DC).

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Predator-induced changes in chemical defences of larval common toads (*Bufo bufo*)

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Many amphibians contain toxins that provide effective defences against predators. Some species sequester toxic substances from their diet, while others are capable of synthesizing such compounds *de novo*. Although there is increasing evidence that chemical defences can be adjusted to the environment, we know very little about predator-induced changes in toxin content and the factors favouring its evolution.

To examine predator-induced changes in chemical defences, we performed a series of experiments in outdoor mesocosms and in the laboratory using tadpoles of the common toad (*Bufo bufo*), which are known to produce toxins themselves. Several experiments delivered negative results: tadpoles did not appear to adjust toxin production in response to predation risk. However, these tadpoles all originated from fish-infested ponds, where failure to produce toxins at a high enough quantity almost certainly results in death. Tadpoles originating from ephemeral ponds where fish are absent exhibited plasticity in toxin production: individuals raised with chemical cues indicating predation risk contained more toxins than their conspecifics that had been raised in a predator-free environment. These results demonstrate predator-induced change in chemical defence and suggest that among-population variation in the inducibility of toxin production is a result of local adaptation to the composition of the prevailing predator fauna.

Effects of a glyphosate-based herbicide on agile frog (*Rana dalmatina*) tadpoles exposed to free-ranging predators

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Glyphosate-based herbicides are among the most widely used pesticides in agriculture. Many previous studies documented lethal or sub-lethal malign effects on larval anuran amphibians. However, an intriguing observation is that phenotypic changes induced by exposure to the herbicide may resemble inducible antipredator responses.

Our aim was to test whether herbicide-induced phenotypic changes may deliver an advantage to tadpoles of the agile frog (*Rana dalmatina*) when facing free-ranging predators. We raised tadpoles in outdoor mesocosms in the presence or absence of the herbicide (0 or 4 mg a.e./L glyphosate) and of predators (empty cage or caged larval dragonfly, *Aeshna cyanea*). After three weeks we observed tadpole behavior, and subsequently removed individuals, photographed them, and transferred them to test boxes, where we performed bioassays using free-ranging predators. After six days, we photographed survivors again, and identified the rearing box, and hence the rearing environment they originated from.

Our results showed that, upon exposure to free-ranging predators, tadpoles that had developed in the presence of predators enjoyed elevated survival probability compared to predator-naïve conspecifics, irrespective of herbicide presence. Importantly, herbicide-exposure during development lead to enhanced survival of tadpoles in bioassays performed in herbicide-free water. However, when we exposed tadpoles to free-ranging predators in herbicide-contaminated water, it was the individuals that first came into contact with the herbicide upon entering the bioassays that had elevated survival. It appears that exposure to the herbicide can affect tadpole survival in complex and non-trivial ways and may even lower mortality rates in amphibian larvae exposed to predators.

Gut microbial diversity is related to host genetic diversity but is not affected by starvation during an el Niño event in Galápagos marine iguanas

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The gut of vertebrates is inhabited by diverse microbial communities, which are essential for their hosts' well-being. Herbivores have a highly fibrous diet rich in complex polysaccharides that cannot be degraded without the enzymes provided by intestinal symbionts. Such is the case of the Galápagos marine iguana (*Amblyrhynchus cristatus*), a unique species that feeds exclusively on algae and harbors specialized bacterial symbionts facilitating digestion. During an El Niño-Southern Oscillation (ENSO) event the amount of grazeable algae is drastically reduced and marine iguanas may experience mortality rates up to 90 %. Despite the pivotal role of gut microbes, the factors shaping their diversity in marine iguanas during el Niño remain unknown.

We sampled all major populations of marine iguanas in an el Niño year to explore whether differences in their microbial communities were related to host genetic diversity or starvation. We found that population differences in microbial diversity were mainly driven by genetic variation, independent of the geographical distance between populations. Populations with high genetic diversity (i.e. heterozygosity, allelic richness and effective population size) harbor a more diverse microbiota than those with low genetic diversity. Body condition also varied among populations but it did not correlate with gut microbial diversity, suggesting a minor effect of starvation on gut microbes. Our study reveals that marine iguana populations with low genetic diversity could be especially vulnerable to el Niño due to their poor diversity of microbes, which could diminish their ability to digest algae, leading to a local extinction risk.

Comparative study of anuran skeletal development in *Duttaphrynus melanostictus* (Bufonidae), *Fejervarya syhadrensis* (Dicroglossidae) and *Microhyla ornata* (Microhylidae) during metamorphosis

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During anuran metamorphosis, ontogenetic changes in chondrocranium, hyobranchial apparatus and axial skeleton could be important to understand their adaptations to various ecological conditions and studying their phylogenetic position (Haas 2003). In India, studies on the skeletal development and ossification patterns for anurans are limited (Saidapur 2001).

Hence I attempted to study the skeletal development and ossification patterns in anurans representing three species from different families: Bufonidae (*Duttaphrynus melanostictus*), Dicroglossidae (*Fejervarya syhadrensis*) and Microhylidae (*Microhyla ornata*) during metamorphosis as their larvae exhibit different feeding habits. Tadpoles at various Gosner (1960) stages were fixed in 10% formalin, followed by staining with the alcian blue-alizarin red double staining method (Dingerkus & Uhler 1977).

Results mainly showed variations in the structure of chondrocranium and hyobranchial apparatus in all the three species, while axial skeleton only showed difference in the onset of ossification. Chondrocranium of *M. ornata* showed C shaped infrarostral cartilage, poorly developed muscular process and absence of suprarostrala ala as compared to other two species. In *M. ornata*, the overall cartilages of the chondrocranium supporting the jaw were poorly developed but showed large and dense branchial baskets for filter feeding. Overall ossification at the completion of metamorphosis is more advanced in *M. ornata* followed by *F. syhadrensis* and *D. melanostictus*. Hence, variations in feeding habits appear to be reflected in ossification patterns and skeletogenesis.

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Genetic diversity and Quaternary range dynamics in Iranian and Transcaucasian tortoises *Testudo graeca*

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Range dynamics of species distributed in temperate zones was significantly influenced by climatic fluctuations during the Pleistocene. While these processes and patterns are relatively well-studied in taxa living in mid and high latitudes of Eurasia, little is known on how glacial and interglacial cycles influenced the distribution of species occupying lower latitudes. The aim of this study was to assess Quaternary range dynamics of the tortoise *Testudo graeca* in Iran and Transcaucasia and to infer how these changes influenced the species genetic diversity. Therefore, we analysed the genetic variability of the cytochrome *b* gene (993 bp) and reconstructed the species' paleogeographic by projecting species distribution models (SDMs) onto paleoclimatic conditions of the Mid-Holocene (6,000 BP) and the Last Glacial Maximum (21,000 BP). We found three mitochondrial lineages in Iran, corresponding to the subspecies *T. graeca armeniaca*, *T. graeca buxtoni* and *T. graeca zarudnyi*, whose current distribution is limited predominately by precipitation. A combination of SDMs and demographic analyses revealed that the ranges of these subspecies experienced only a slight shift during the Quaternary and did not reveal significant contraction since the LGM. Long-term survival of *T. graeca* in Iran and Transcaucasia led to high genetic diversity, especially in the vastly distributed *T. graeca buxtoni*. These results indicate that range dynamics of ectothermic taxa occupying lower latitudes in the western Palaearctic might be more complex and may not follow a simplistic scenario of glacial retraction and postglacial expansion.

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A matter of proportion? Associational effects in a tadpole-fish predator system

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Prey that possess chemical defences are avoided by the predators, but this avoidance can be mediated by neighbouring species (Underwood et al. 2014). We evaluated the presence of such associational effects in tadpole communities, suggested by Nelson et al. (2010, 2011) and Hartman et al. (2014), using a gradient of relative abundance of tadpoles of two anurans differing in palatability to vertebrate predators: common toad *Bufo bufo* (BB) and common frog *Rana temporaria* (RT). Tadpoles were kept in varying relative abundances (0:40, 2:38, 10:30, 20:20, 30:10, 38:2, 40:0) at constant total initial density (40 tadpoles/cage), either with or without omnivorous fish (carp *Cyprinus carpio*) that had constant access to alternative food (fish feed pellets). Tadpoles were raised until metamorphosis.

Fish reduced survival of both toad and frog tadpoles, the effect being markedly stronger for frogs (Generalized linear mixed models; BB: $F=7.59$, $P=0.024$; RT: $F=36.20$, $P<0.001$). However, in fish presence both BB and RT survived better with increasing proportion of BB per cage (BB: $F=5.26$; $p=0.044$; RT: $F=7.21$, $p=0.024$). RT mass at metamorphosis was lower in tadpoles exposed to fish ($F=16.67$; $P<0.001$), but larger in communities dominated by BB ($F=18.10$; $P<0.001$). In toads, the effect of fish presence was not significant, but, surprisingly, metamorph mass was positively affected by increased toad tadpole density ($F=19.20$; $P<0.001$).

The results suggest that during the larval stage, palatable tadpoles (RT) are able to enjoy some degree of associational protection from the presence of unpalatable tadpoles (BB). In contrast, the effectiveness of the chemical protection of the latter can be reduced by the prevalence of palatable prey.

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Embryology of the vomeronasal organ and associated structures in the brown anole (Squamata: Iguania)

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The traditional morphological division of Squamata is substantiated on the grounds of similarities of many morphological features between the two groups Iguania and Scleroglossa. It is very easy to explain it, referring to the tongue anatomy and vomerofactory abilities.

Iguania are characterized by many plesiomorphies. Scleroglossa are variably specialized for vomeronasal chemoreception. Such taxa as snakes are characterized by well developed sensory epithelium and slim bifid tongue. Iguanian lizards rely mainly on visual prey discrimination. They hunt by means of their fleshy tongues, which are only nearly incised on the tip. The Vomero Nasal Organ (VNO) seems to be weakly developed and its duct is confluent with the choanal groove leading to the internal naris posteriorly. The brown anole (*Anolis sagrei*) as a member of Iguania is considered to be a visually oriented predator. Moreover, it represents trunk-ground ecomorphs. Thus, at least by the fact of phylogenetic position and arboreal lifestyle, the VNO should be weakly developed. However some studies suggests that vomeronasal chemoreception may still be important in this species. Our study aims to analyse embryonic development of the VNO and associated structures (such as the nasal cavity, choanal groove and vomeronasal nerve) of the brown anole utilizing both, 3D visualisation, on the basis of micro CT imagining, and transmission electron microscopy.

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Drivers of evaporative water loss in Podarcis lizards from Greece

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Evaporative water loss (EWL) is an ecophysiological trait that receives considerably less attention than temperature in lizard biology. Since organisms function within certain limits of each physiological variable, the role of water in small ectotherms with low homeostatic capacity is vital (Carneiro et al. 2017). Apart from being substantial on organismal biochemistry, there is recent evidence that water may be a more important limiting factor in the distribution of lizards than the traditionally examined temperature (Carneiro et al. 2017) and, as such, it may be a more significant predictor in species distribution modelling and conservation (Carneiro et al. 2015). The drivers of EWL patterns though remain poorly understood. In this study we compare the EWL rates and patterns in seven lizard species (*Podarcis cretensis*, *P. erhardii*, *P. gaigeae*, *P. muralis*, *P. peloponnesiacus*, *P. siculus* and *P. tauricus*) from various habitats in Greece and attempt to interpret the observed differences taking into account environmental variation and phylogeny (Garland et al. 1993). Our results indicate that the pattern of EWL through time (instantaneous EWL index, Osojnik et al. 2013) remains relatively constant and is similar between species. On the other hand the amount of water lost (accumulated EWL index, Osojnik et al. 2013) varies between species and differences become more pronounced as time progresses. Preliminary results from comparative phylogenetic analysis (Garland et al. 1993) indicate that the observed variation is not due to phylogeny, but may be attributed to environmental variation.

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Phylogeny and phylogeography of the Roughtail Rock Agama *Stellagama stellio*

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The Roughtail Rock Agama, *Stellagama stellio*, is distributed in the E. Mediterranean at the crossroads of three continents (Valakos et al. 2008). The species' high morphological variation along this range has led to the description of seven subspecies: *S. s. stellio* in Greece, Turkey, Syria, Lebanon, Israel, Jordan, *S. s. daani* in Greece and Turkey, *S. s. cypriaca* in Cyprus, *S. s. vulgaris* in Egypt, *S. s. brachydactyla* in Jordan, Saudi Arabia, Sinai, Israel, *S. s. picea* in Jordan, Syria, Saudi Arabia and *S. s. salehi* in Sinai, Israel.

We explored the evolutionary history of the species for the first time, using >150 individuals from the whole range of its distribution. Two mitochondrial (ND4, 16S) and three nuclear (NKTR, CMOS, MC1R) genes were targeted and phylogenetic (Maximum Likelihood and Bayesian Inference), species-tree (*BEAST in BEAST2) and phylogeographic / cladochronological (BEAST2, Bouckaert et al. 2014) approaches were implemented.

Stellagama stellio is a well-supported monophyletic species that diverged during the Messinian. Five of the seven major clades found generally correspond to described subspecies, while two do not fit to any currently named taxon and include populations of southeast and southwest Anatolia. Moreover, in view of the phylogenetic and the cladochronological results, we propose the elevation of the Cypriot population to the species' level. The major biogeographical events shaping the species' distribution are the uplift of the Amanos Mts, the filtering effect of the Taurus Mts and the Messinian Salinity Crisis.

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Revealing high-risk ponds of the chytrid fungus in Swedish amphibian populations

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Batrachochytrium dendrobatidis (*Bd*) has caused decline or extinction of more than 200 anuran (frogs and toads) species globally. Its occurrence in northern Europe is not well-known. In 2015 and 2016, we sampled 859 breeding amphibians from 31 ponds in Skåne. We found that ca. half of the ponds (and all six species) were infected by *Bd*. The main objective of the present study is to understand how pond characteristics, urbanisation and surrounding landscape at three different spatial scales (500 m, 2000 m and 5000 m) affect the distribution and infestation risk of *Bd*. The results show that pond size, surrounding ponds, pH and species composition increase the risk of *Bd*. The area of sea water in the landscape are also affecting the distribution of *Bd*, probably due to the coast-living and highly infected green toad. The most important factors were the focal pond size and surrounding ponds, where the 2000 m scale explained more variance than the 500 and 5000 m scale. These results can identify high-risk ponds and have possible conservation implications to counteract the spread of *Bd*.

Let's save the amphibians, but how? A case conservation study of *Lyciasalamandra* species.

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The genus *Lyciasalamandra* is endemic to the southwestern coast of Turkey and nearby Aegean Islands (including Kastellorizon, Karpathos, Saria, and Kasos, in Greece). Currently seven species have been described within the genus. All *Lyciasalamandra* species are classified under the IUCN categories CR (critically endangered) or EN (endangered). They are currently under threat mainly by habitat destruction and degradation, caused by forest fires and deforestation. On the other hand there is another new threat arising. It is uncontrolled overharvesting of the specimens for scientific studies by national and international scientists in last decade. Despite the national and international laws and guidelines regarding the research and scientific collecting of threatened species, such scientific progress is unfortunately ongoing. Here we highlight and discuss the current taxonomical progress on *Lyciasalamandra* and provoke ideas for new molecular methods for the conservation of *Lyciasalamandra*.

The evolutionary history of Mediterranean amphibians - a comparison of different calibration approaches

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Our knowledge of the geological history of the Mediterranean region is without comparison, especially for its western part surrounding the Alboran and the Thyrrhenian Seas. We reconstruct the evolutionary history of all amphibian species of the Mediterranean region in a Bayesian framework. Fossil records usually provide suitable information for molecular clock calibration, but since they are rare for the Mediterranean, we conducted a two-step approach for divergence time estimation. Firstly, we calibrated a family and genus tree for western Palearctic amphibians and numerous outgroup taxa based on four nuclear genes, five mitochondrial genes and a combination of both using 12 widely accepted fossil calibration points for amphibian evolution. Secondly, we applied an evolutionary timeframe on species level based on the five mitochondrial genes only and on the divergence time estimates inferred from the primary calibration of the nuclear family and genus tree. The primary calibration based on the mitochondrial genes produced much older divergence time estimates than the nuclear genes and the combined dataset for the family and genus tree. Since evolutionary scenarios strongly depend on the data used and the methods applied, every single analysis will inevitably result in a unique evolutionary timeframe. Hence, we can easily infer several dated evolutionary scenarios for all Mediterranean amphibian species; however, it is not possible to decide which one is true.

Hybridization patterns in two contact zones of grass snakes reveal a new Central European snake species

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Recent studies found major conflicts between traditional taxonomy and genetic differentiation of grass snakes and identified previously unknown secondary contact zones. Until now, little is known about gene flow across these contact zones.

Using two mitochondrial markers and 13 microsatellite loci, we examined two contact zones. One, largely corresponding to the Rhine region, involves the western subspecies *Natrix natrix helvetica* and the eastern subspecies *N. n. natrix*, whereas in the other, more easterly, contact zone two lineages meet that are currently identified with *N. n. natrix* and *N. n. persa*. This second contact zone runs across Central Europe to the southern Balkans.

Our analyses reveal that the western contact zone is narrow, with parapatrically distributed mitochondrial lineages and limited, largely unidirectional nuclear gene flow. In contrast, the eastern contact zone is very wide, with massive nuclear admixture and broadly overlapping mitochondrial lineages. In combination with additional lines of evidence (morphology, phylogeny, divergence times), we conclude that these differences reflect different stages in the speciation process and that *Natrix helvetica* should be regarded as a distinct species.

Comparative analysis of skin bacteriome from four natural populations of *Proteus anguinus* in Slovenia

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Beside its ecological role as a top predator of underground waters of the Dinaric Karst and host of unique parasites, the interactions of the olm, *Proteus anguinus*, with other organisms and microorganisms in its natural environment are practically unknown. Due to the recognized importance of animal microbiomes as a source of metabolites and protection from pathogens, we investigated the skin of this troglomorphic amphibian for origin, specificity and potential role of its bacteriome.

Skin swabs from individuals belonging to four populations of *Proteus* in Slovenia were analyzed by IonTorrent next generation sequencing of their 16S rRNA genes. The obtained bacteriomes were compared within and between populations, as well as to the water bacteriome from one of the collecting sites.

All skin bacteriomes exhibited considerably lower bacterial diversity compared to water, and their significant portion, comprising known taxa of environmental Alphaproteobacteria, Betaprotetebacteria and Actinobacteria, was shared between all *Proteus* populations. These results indicate the selective pressure of the skin microenvironment to colonizing bacteria and facilitation of selected bacterial groups, representing normal skin microbiota. While only moderate shifts in the bacteriome of the same individual between consecutive years were observed in unpolluted environment, a replacement of a considerable portion of normal microbiota with Enterobacteria has been detected in an individual exposed to sewage and farming pollution. Presence of normal skin microbiota provides the first step towards understanding its role and mechanisms in proteus' extended phenotype and/or protection of its delicate neotenic skin against omnipresent and newly emerging pathogens.

How does the pancreas of snakes differentiate?

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The pancreas is an important glandular organ present in all vertebrates species but literature review indicated that little is known of this organ in reptilian species. For this reason we described the structure and ultrastructure of differentiating pancreas in grass snake (*Natrix natrix*) embryos. The primordium in the grass snake is formed by two buds – a dorsal and a ventral one – that are not connected until the end of stage II. This distinguishes them from the majority of vertebrates. The gall bladder of these embryos is connected with the liver only by a thin cystic duct, which differs from other vertebrates. At developmental stage IX, the pancreas of the grass snake penetrates the ventral part of the developing spleen and divides it into three parts. This is unique among vertebrates. At the end of the embryonic development, the pancreas, the spleen and the gall bladder form the so-called triad. The embryonic endocrine pancreas of grass snake contains the four main types of the endocrine cell types (A, B, D and PP). The order of the differentiation of the granules within the main endocrine cells in the grass snake embryos is different from other vertebrates because maturation of the granules depends on the nutrition of the embryo. Within their granules different morphological subtypes could be distinguished, that indicated their maturity, which may be related to the different content of these granules during the process of maturation.

Approvals for research were obtained from the Local Ethics Commission in Katowice (41/2010; 87/2015), the General Directorate for Environmental Protection of the Polish Ministry of Environment (ref. No: DOPozgiz-4200/II-88/4189/10/JRO) and the Regional Directorate for Environmental Protection in Katowice (WPN.6401.257.2015.DC)

The first records of *Batrachochytrium dendrobatidis* in a Latvian Zoo

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The amphibian fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) has not been detected in Latvia in a previous screening (Ouellet et. al. 2005), and no screening for the recently discovered salamander pathogen, *Batrachochytrium salamandrivorans* (*Bsal*) detection has so far been carried out in this country. Invasion of chytridiomycosis, among other mechanisms, is mediated by movement of exotic species, therefore collections of tropical amphibians in Zoos are at risk.

Samples were collected in 2017 from two main zoos in Latvia. We analysed 60 individuals (31 species) from Riga Zoo and 40 individuals (7 species) from Latgales Zoo. *Bd* infection was identified with quantitative real-time PCR (qPCR) amplification following the protocol of Blooi et al. (2013). We did not detect *Bsal* in any sample. *Bd* was detected in 5 of analysed 60 samples (8.3%) from Riga Zoo: *Tylototriton verrucosus* (1 sample, 339.25 GE), 2 *Bombina bombina* (2 samples, 339.25 GE), and in *Pelophylax lessonae* (2 samples, 0.7 GE, 1.25 GE).

The *Bd* infection of *B. bombina* in Riga Zoo is an alarm signal highlighting the risk of dissemination of the pathogen to wild and among the captive collection. The Riga Zoo has for many years bred and later released *B. bombina*, *Hyla arborea*, and *Epidalea calamita* without checking for infection by chytridiomycosis. Further research on the distribution of *Bd* and *Bsal* in Latvia is necessary.

The study was supported by the Administration of Latvian Environmental Protection Fund (Project 1-08/153/2017).

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The evolution of parental investment in caecilian amphibians

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Among amphibians, caecilians (Gymnophiona) exhibit a considerable variation in reproductive modes, including both oviparity and viviparity, combined with highly unusual investment strategies. The current knowledge on the reproductive modes was integrated into a comparative phylogenetic analysis of the evolutionary scenario of parental investment in caecilians. Phylogenetically basal caecilians possessing a biphasic life cycle that includes an aquatic larval stage invest into larger eggs, whereas some phylogenetically derived caecilians (i.e. the Teresomata) have a smaller clutch size and show a reduction to either medium-yolked (mesolecithal) or small-yolked (microlecithal) eggs. Via alternative pathways of parental investment, such as intrauterine feeding in viviparous taxa and maternal dermatotrophy in oviparous taxa, teresomatan caecilians increase both offspring size and quality. However, more data regarding reproductive biology are needed to obtain a fully resolved understanding of the evolution of reproduction in caecilian amphibians.

Mechanisms to avoid fish as predators in stream side salamander larvae (*Salamandra infraimmaculata*)

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Fire salamander are rarely reported to co-exist with fishes. One exception is the Tel Dan Nature Reserve (hereafter TD), a permanent brook system, where aquatic larvae of *Salamandra infraimmaculata* are sharing the habitat with native fish. We approach the strategies that help the larvae to survive, applying a series of experiments. Adult females have strong abilities to select the breeding habitat. Therefore, we tested 23 gravid females for their preferences for a fish free environment to deposit their larvae. The larvae were deposited randomly (one sample t-test, $df=15$, $t=0.39$, $p=0.70$) but spread over multiple pools and nights. Additionally, larvae from TD and Kaukav, a naturally fish free site were reared in a fish cue treatment and a fish free control treatment. Larvae from TD, showed reduced growth in presence of fish, whereas no such effects were present for larvae from Kaukav (LME_{growth rate}: treatment, $F_{1,64}=4.51$, $p=0.038$, sites $F_{1,6}=1.04$, $p=0.35$; treatment * site, $F_{1,64}=4.57$, $p=0.036$). In the third experiment naïve larvae from different (fish / fish-free) populations, showed a higher probability to hide, if they were faced to fish cues for 10 minutes, than in the fish cue free control GLMER_{hiding} ($df= 84$, treatment: $F_1=8.85$, $p=0.03$, age $F_1=3.94$, $p=0.05$). Taken together we found developmental and behavioural reactions of larvae in response to fish cues, which were most pronounced in larvae from sites co-existing with fish in the wild. Likely co-evolution with fish in TD induced a change in behaviour that allows the larvae to survive with predation pressure.

Biogeography of the viviparous lizard, *Zootoca vivipara* LICHTENSTEIN, 1823) (Squamata: Lacertidae) in central Europe and possible re-colonization of the Baltic Sea basin and the Balkans: new karyological evidence

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Based on chromosomal and mtDNA data several distinct chromosomal forms and haplotypes of the viviparous lizard *Zootoca vivipara* (Lichtenstein, 1823) have been described from central Europe.

We studied for the first time the karyotype of specimens from localities in northern and southern parts of central Europe – the Baltic Sea area (western Pomerania and northern Brandenburg / NE Germany) and central Balkans (Mt. Kopaonik, Serbia). All individuals karyotyped represent the viviparous western form of *Z. v. vivipara*. We demonstrate that this form inhabits the western part of the south coast of the Baltic Sea between the German harbour city of Kiel in the west and the Russian harbour city Kaliningrad in the east, whereas the other, so-called Russian form of *Z. v. vivipara* with a different karyotype inhabits the eastern part of this coast. At the southeasternmost margin of central Europe (Serbia) we documented the western form.

Together with previous data sets, our results suggest a wide distribution of the western form in central Europe. The present chromosomal information confirms (1) karyological similarity of the central Balkans and western, central and partly eastern parts of Carpathian basin fauna, (2) possible ways of re-colonization of the Balkans by the western form of *Z. v. vivipara* and of the Baltic Sea basin by two forms of *Z. v. vivipara* and (3) a localization of a secondary contact zone between these forms in the latter.

Long term population trends of *Bufo bufo* and *Rana temporaria*: An analysis of two decades amphibian fencing in Salzburg

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This study focusses on the population trends of two widespread European Anuran species: *Bufo bufo* and *Rana temporaria*. The basis of this study are data gathered over two decades of amphibian fencing alongside roads in the Austrian state of Salzburg.

Different statistical approaches were used to analyse the data: calculation of an average locality index as well as the statistical software TRIM.

The results show differing overall trends for the two species: *Bufo bufo* being stable, while *Rana temporaria* showing a substantial decline over the last two decades. Further analyses based on geographic categorization reveal the strongest decrease in the alpine range of the species.

Drainage and agricultural intensification are still ongoing problems within alpine areas, not only in Salzburg. Particularly in respect to micro-climate and the availability of spawning places these changes have a higher impact on the habitats of *Rana temporaria* compared to *Bufo bufo*. Therefore we consider habitat destruction to be the main reason behind this dramatic decline. We also conclude that the substantial loss of biomass of a widespread species such as *Rana temporaria* must have a severe and often overlooked ecological impact.

Complex impact of goldfish introduction on palmate newt dominated pond ecosystem

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Introduction of invasive species is one of the main threat to amphibian populations. Beyond direct predation or competition effects with native species, omnivorous invasive species are of particular concern as they can have important and long-term impacts on native populations by affecting the entire community. In Larzac (Southern France), declining trends in the pond-breeding palmate newt populations are correlated to goldfish (*Carassius auratus*) introductions (Denoël & Lehmann, 2006; Denoël & Winandy, 2015). However, the processes leading to local extinction of newts from invaded ponds are not fully understood. In particular, goldfish seem to have a strong impact on the pond ecosystem, potentially making it an unsuitable habitat for newts.

To assess the impact of goldfish leading to newt extinction, we compared the community assemblages and modelled the communities' isotopic niches of ponds naturally dominated by the palmate newt (*Lissotriton helveticus*) and ponds where these newts have been extirpated following goldfish introduction, using varied techniques including Carbon and Nitrogen stable isotopes analyses in a Bayesian framework.

Our results show that beyond direct interactions with newts, goldfish have profound detrimental impacts on the aquatic vegetation, anurans, macroinvertebrates and zooplankton communities of the ponds by operating a global alteration of the food web on multiple trophic levels, reducing its size, diversity and evenness, and consuming almost all exploitable resources available for newts. In the long-term, these changes likely explain newts' exclusion from invaded ponds and illustrate the detrimental effects of goldfish introductions for native ponds ecosystems.

This research was supported by Fonds de la Recherche Scientifique – FNRS.

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Reproductive success of the European pond turtle (*Emys orbicularis*) on Ljubljana marshland

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Agriculture presents one of the major conservation threats to reptiles, especially those confined to land habitats. European pond turtles (*Emys orbicularis*) spend most of their lives inside or in the vicinity of fresh-water bodies, but females come on land to bury their eggs in the soil.

We assessed the reproductive success of *E. orbicularis* in the Ljubljana marshland, Slovenia, where they live in water ditches surrounded with agricultural fields. Previous studies indicated lack of juveniles in this population during the past decade. In 2015-2017 we captured turtles using traps to estimate the age and sex structure of population. Additionally, we have equipped ten females with VHF telemetry tags to locate and monitor nests. We gathered data on egg deposition, egg survival and hatchling success for at least eleven nests. After locating the nests, we mechanically protected them with wire mesh, which was 100% successful in preventing predations. Eight females deposited eggs once or twice from early-June to mid-July, but reproductive success rate was almost zero. In five nests eggs were mechanically damaged due to ploughing and in six nests the cause for hatching failure could not be determined. Only one nest has been partly successful (two out of eight hatchlings successfully left the nest). These results reveal lack of effective measures to ensure successful reproduction of this species, which faces extinction in this protected area if the situation does not improve.

Amphibian adaptation to extreme environment *in statu nascendi*: water frogs and brown frogs in cold climatic conditions

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The formation of the first populations of *Pelophylax ridibundus* in the middle Kamchatka was the result of single successful introduction by humans in the late 1980-s. At the present time, more than 20 localities were revealed in Kamchatka, most of them – in habitats with high supply of warm water all year round. In these populations, up to 8 offspring cohorts were recorded during a year. But in habitats with minimum warm water supply, only one cohort was revealed within each generation. In the Middle Ural, the newly introduced populations of this species were formed in warm waters only. Besides two summer cohorts, one overwintering cohort with metamorphosing in spring of next year developed. Interestingly, a small overwintering cohort was revealed in one population which inhabited “non-warmed” waters.

The unique *Rana temporaria* population in southern Kamchatka originated from releasing 150 immature frogs. After 10 years, the numbers of breeding females was already 2,648! The activity season duration at the release locality (4 months) is 2 months shorter than that at the original locality of the translocated frogs from Moscow district. Female age at first reproduction is 3 or 4 years, both sexes demonstrate relatively slow postmetamorphic growth and low body length at each age. The presence of a small pool warmed by thermal waters (along with numerous unwarmed ones) and changes in the relationship between larval growth and development promoted this successful introduction.

This study was funded by the Russian Foundation for Basic Research, project No 16-04-01771.

Genetic diversity of *Vipera berus nikolskii* and *Vipera renardi* in Eastern Ukraine and Southern Russia

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Genetic diversity in six populations of *V. b. nikolskii* and *V. renardi* from Eastern Ukraine and Southern Russia was assessed with 12 microsatellite loci. Samples of 4 to 18 individuals per population represent comparatively abundant and continuous populations except of one locality of *V. renardi* from Matiashivka vill. vicinities, Poltava region, Ukraine, where snakes live on heavily isolated, small habitat and individuals demonstrate unusual coloration and high proportion of rare morphological characters states. All twelve microsatellite loci adapted from previous *V. berus*, *V. eriwanensis* and *V. ursinii* studies have passed standard quality control and did not deviate from H-W equilibrium, have no evidence of scoring errors, null-alleles or physical linkage.

Basic genetic diversity measures show high diversity of most studied populations. In *V. b. nikolskii* observed heterozygosity exceeds 0.6 in two populations from the core of taxon range and was equal to 0.476 in the population from the periphery of range in Tula region, Russian Federation. For the second species, *V. renardi*, two populations has H_o 0.52 and 0.57, while the third, from morphologically deviant and small Matiashivka population, has $H_o=0.262$, almost two times lower than H_e , significantly different from zero F_{is} , lowest amount number of private alleles and mean allele diversity, adjusted to the sample size. Low genetic diversity and evidence of inbreeding may explain unusual coloration and morphology of this population.

Phylogeography and adaptive evolution of the Caspian whipsnake (*Dolichophis caspius*)

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The Caspian whipsnake occurs across South-eastern Europe and Central Asia and is adapted to different environments. Over 120 tissue samples from nine Eastern and Central European countries were collected noninvasively and used for DNA extraction. Double-Digest Restriction Associated DNA (ddRAD) sequencing of 96 samples with high DNA quality provided 21K single-nucleotide polymorphisms (SNPs) to estimate genetic distances between snakes of different localities.

Based on analysis of population structure and selection of Principal Components showing most variation, we defined eight main groups: 1- Croatian islands (Olib, Lastovo); 2 - Serbia (Banja, Zlot), Hungary (Danube banks), Croatia (Baranja); 3 - Hungary (Villány); 4 - Hungary (north Budapest); 5 - Albania, Macedonia, Serbia (Cukarka); 6 - Bulgaria, Greece (Crete, Loutros); 7 - Greece (Samos), Turkey; and 8 - Ukraine. We found that snake populations from the Croatian islands of Lastovo and Olib are highly inbred, likely due to a small founder population. The high differentiation between the three Hungarian populations could be explained by multiple colonisation events, and recent geographic barriers. Nowadays the Danube provides a dispersal system through small bank cracks, while the other two populations, located further away from the river were probably colonised during an earlier event.

Integrating micro-scale analyses in the study of niche similarity: preliminary results in the hybrid zone between *Hydromantes italicus* and *H. ambrosii*

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Quantification of niche similarity may be an important instrument to test whether hybrid populations have niches that are similar or intermediate to their parental species, or instead occupy transgressive niches. However, the evolution of niche is often studied using macroecological data with a coarse resolution. European cave salamanders (*Hydromantes ambrosii*, *H. italicus* and their hybrid populations) are an excellent experimental model to perform fine- scale assessment of niche differences between parental species and their hybrids of the species

In June-July 2012-2016, we surveyed 138 natural and artificial caves. We subdivided each cave in 3-m longitudinal sectors, in which we detected active salamanders and measured microhabitat features (humidity, air temperature and incident light). We then used the PCA-env approach to assess niche shifts between pure and introgressed salamander populations, we calculated niche overlap using the Schoener's *D* statistics and we used equivalency tests to assess the significance of niche differences between taxa.

Pure *H. ambrosii* populations were linked to dark and cold underground sectors, while pure *H. italicus* populations chose dark and wet sectors. *H. ambrosii* introgressed populations showed a significant ($P=0.001$) expansion toward warmer, drier and more luminous conditions compared to the non-introgressed populations. Introgressed *H. italicus* showed a significant niche shift ($P=0.006$), with expansion toward conditions with lower humidity and more light compared to *H. italicus*.

Our results are consistent with the transgressive niche hypothesis: hybrids have a broader ecological niche than the parental species, allowing them to exploit environments with harsher conditions.

Seasonal T-level fluctuations and protein content of femoral gland secretions in the common wall lizard (*Podarcis muralis*)

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Lizards have a series of epidermal glands around the pre-cloacal region, which produce secretions involved in intraspecific signalling (Martín & López, 2015). Their activity is controlled by testosterone (T) (Padoa, 1933; Baeckens et al., 2016) and fluctuates seasonally (Padoa, 1933; Alberts, Pratt & Phillips, 1992). Secretions are made of proteins and lipids (Alberts, 1990), which may carry different information (Mangiacotti et al., 2017), being lipids related to the emitter's quality (López, Amo & Martín, 2006), whilst proteins to its identity (Alberts, 1992). Coupling the above hypothesis with the testosterone role, we may predict that higher T-level should correspond to higher secretion amount and to particular enrichment in its lipophilic fraction at expense of the proteins. We verified this prediction in a natural population of *Podarcis muralis* from northern Italy.

From March to October 2016 where we monthly collected blood and secretion samples from different males (N=174). Secretions were weighted, the protein content quantified (BCA assay), and haematic T-level measured (Radio-Immuno-Assay).

The highest T-level was detected in March (93.57 ± 14.87 ng/ml), it gradually decreased till August (2.22 ± 0.28 ng/ml), and started again to rise afterwards. Secretion mass peaked in April (2.05 ± 0.19 mg), slightly decreased till July (1.40 ± 0.16 mg), and abruptly fell from August onwards (0.40 ± 0.04 mg). The protein fraction represented half of the secretion mass ($50.7 \pm 0.6\%$) until July, but was rising up to 75.8% ($\pm 3.7\%$) in August-September. As expected, during the reproductive season, males enrich the costly part of the secretion, while maintaining the protein part (identity) of the signal.

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Diversity and Distribution of the Amphibians and Reptiles of Angola: turning historical records into an Atlas of the Angolan herpetofauna

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Angola is one of the largest countries in sub-Saharan Africa, but remains one of the least known in terms of its biodiversity. Research focused on Angolan herpetology has been sparse throughout the last two hundred years, and came to a complete halt during the civil war that afflicted the country from 1975 to 2002. In order to support new and ongoing field research in the country, we compiled all the known bibliographic records published from 1840 to 2017. These data were critically reviewed, taxonomically and nomenclaturally updated and georeferenced.

A total of 389 taxa (116 amphibians and 273 terrestrial reptiles) were confirmed to occur in Angola, although many others are expected to occur. Our results also show that the coverage of distributional data for Angola is biased towards certain provinces, with some others having a very limited amount of data. The available information provides a preliminary estimation of the biogeographic patterns of the country, which forms a crucial piece of the sub-Saharan African zoogeographic puzzle, with a clear turnover of Western/Central African to Zambezian/Southern African faunas. Traditional divisions of Angola into northern and southern regions of endemism are largely supported and the Angolan Escarpment and the Kwanza River Basin are also revealed as distinctive.

Comparative evolutionary history of the Western Mediterranean vipers, *Vipera aspis* and *V. latastei-monticola*

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Past paleogeographical and climatic events shaped biodiversity patterns of the Western Mediterranean Basin. Ectothermic physiology and particular life history traits of vipers (Viperidae) make them highly susceptible to range shifts and demographic processes driven by climate changes. Previous works have identified genetic structure for the Western Mediterranean vipers, *Vipera aspis* and *V. latastei-monticola*; however, the ecological processes that might have triggered their diversification remain poorly understood.

This work aims to compare the evolutionary history of both species, combining phylogeographic, paleoclimatic and ecological niche analyses. Specifically we compare, for species and main lineages: (1) genetic structure and diversity, and time of divergence; (2) location of refugia and responses to Pleistocene climatic oscillations; and (3) ecological niches. Bayesian inferences were performed over mitochondrial and nuclear sequences (four genes) from 351 specimens, covering distributional ranges of both species. Paleoclimatic reconstructions and ecological analyses were based on 4,056 species records and climatic variables. Mitochondrial structure is deeper in *V. latastei-monticola* than in *V. aspis*, evidencing a late Miocene-Pliocene diversification process in the former species. Nuclear genes showed low variability and incongruence compared to mitochondrial genes. Paleoclimatic reconstructions suggest that species and major lineages likely responded differentially to warm and cold periods of the Pleistocene. Climatic stable areas likely acted as refugia since mostly fit genetic diversity of species. Ecological tests showed reduced niche overlap and high relation to geographic regions. Our multidisciplinary approach allows proposing scenarios for the evolution of these species that might be representative of other Western Mediterranean taxa.

Physiological plasticity in a successful invader: rapid acclimation to cold occurs only in cool-climate populations of cane toads (*Rhinella marina*)

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Physiological plasticity may facilitate invasion of novel habitats; but is such plasticity a pre-adaptation, or is it elicited only by specific climatic challenges? In Australia, the ability of invasive cane toads (*Rhinella marina*) to penetrate into severely cold areas has been facilitated by an ability to rapidly acclimate to cool conditions.

To investigate whether this physiological plasticity is found in invasive cane toads in all populations or is only seen in cool climates, we measured the acclimation ability of toads from warm and cool populations across Australia and Hawai'i. We collected toads from the field and placed them at either 12 °C or 24 °C for 12 hours before measuring their righting-response (as a proxy for critical thermal minimum, or CTmin). Toads from the coolest region (NSW) demonstrated plasticity as expected, with exposure to 12 °C (vs 24 °C) decreasing CTmin by 2 °C. In toads from other Australian populations, CTmins were unaffected by our thermal treatments. Hawai'ian toads from a cool, wet site also rapidly acclimated to cool conditions, whereas those from warmer and drier Hawaiian sites did not.

This result demonstrates divergence in thermal plasticity among populations of invasive cane toads, with rapid thermal acclimation manifested only in two cool-climate populations from widely-separated sites. Predictions about the potential range and ecological impact of invasive species must take into account the possibility of geographic heterogeneity in levels of physiological plasticity; data from other parts of the species' range may fail to predict levels of plasticity elicited by thermal challenges.

Anatolian Vipers - comparing IUCN Red List Data and state of knowledge – *Vipera eriwanensis*, *V. darevskii*, *V. anatolica*, *Montivipera (bulgardaghica) albizona*

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The information displayed in “The IUCN-Red List of Threatened Species” for Anatolian vipers goes back to assessments made in 2008 with three taxa classified as CRITICALLY ENDANGERED: 1) *Vipera darevskii*, 2) *Montivipera wagneri*, 3) *V. anatolica*; two as ENDANGERED: 4) *M. (bulgardaghica) albizona*, 5) *V. kaznakovi*; one as VULNERABLE: 6) *V. eriwanensis*; three as NEAR THREATENED: 7) *V. (berus) barani*, 8) *M. raddei*, 9) *V. (ammodytes) transcaucasiana*; two as LEAST CONCERN: 10) *M. b. bulgardaghica*, 11) *M. xanthina*; and one not classified or threatened: 12) *Macrovipera lebetina*.

These IUCN data are often based on insufficient to misleading and unrealistic information, relating to population sizes and smuggling for the international pet trade. Unfortunately, such mis-information can be counterproductive when it comes to direct conservation and funding efforts, affecting international collaborations and permit requests in Turkey, as well as finding its way into recent publications on the conservation status of these vipers. Herein in part 2, I present an updated summary on the distribution and threat statuses of three Anatolian dwarf vipers (*Vipera eriwanensis*, *V. darevskii*, *V. anatolica*), and one Mountain Viper (*Montivipera [bulgardaghica] albizona*). We gathered information over the last three years from personal field tours, articles, colleagues, and public online sources (citizen science, news, websites, etc.). The collected data show or indicate a vastly different situation of these Anatolian vipers than previously assessed and displayed in the IUCN Red List files.

Snakes on Montecristo Island, Italy: Dwarfism and resource partitioning

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The small rocky Mediterranean island of Montecristo, Italy, is characterized by harsh environmental conditions and reduced biota, a good platform to study rapid selective processes.

We measured body size (SVL) and recorded diet of the two island snake species, the Asp Viper (*Vipera aspis*) and the Western Whip Snake (*Hierophis viridiflavus*), and compared these data with populations of conspecifics from the mainland.

Three principal results/comparisons are: (1) no new niche shift regarding food or habitat for insular snake species compared to mainland populations and a similar taxonomic diet composition between both insular species (lizards and birds), though a greater trophic niche breadth for whip snakes (i.p. more anurans and invertebrates). Both species yield a similar insular distribution and habitat use, but whip snakes occupied open areas more frequently; (2) significant body size shift (insular dwarfism) of the whip snake by 30% (mean insular SVL ~70 cm vs. mean mainland SVL ~105 cm), and close to 20% in the viper (mean insular SVL ~45 cm vs. mean mainland SVL ~54 cm); and (3) hunting shift in vipers with 21% of individuals found in arboreal ambush position with neck and head suspended in the air and tail anchoring the entire body to the branch. Bird hunting is an alternative strategy compared to mainland populations (foraging for mice) possibly to compensate for the lack of suitable micro-mammals on Montecristo Island. In conclusion, stringent conditions on an island exert variable effects on snake species, depending on the selective forces, species-specific requirements and plasticity.

To bite or not to bite? Cannibalism ecology in different fire salamander populations

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Intraspecific predation (cannibalism) often constitutes a conditional strategy that can offer adaptive advantages where resources are scarce. Cannibalism is a plastic behaviour, and can be affected by environmental pressures. Among them, predation risk and prey availability represent key factors heavily influencing cannibalistic dynamics.

The fire salamander, *Salamandra salamandra*, is a widespread epigeal urodele which in some cases also breeds in caves. These habitats constitute a refuge environment where larvae find favourable conditions, such as constant environmental features and predator absence. However, in caves food resources are extremely scarce, and food deprivation might stimulate cannibalistic interactions. In this study we evaluated the influence of predation risk and habitat of origin on larvae cannibalistic tendency. We collected newborn larvae from caves and outdoor streams, and reared them under different risk conditions. The aggressive behaviour of larvae toward conspecifics was measured in behavioural trials. During trials, larvae were also exposed to different combinations of the chemical cues of predators and wounded conspecifics. The presence of predators strongly decreased aggressiveness. Moreover larvae from caves exhibited a more plastic behaviour than larvae from streams and, interestingly, they strongly increased aggressive displays when exposed to wounded conspecific cues.

This study highlights the complex tangle of variables driving intraspecific predation and how species plasticity and environmental pressure can determine the expression of diverse behaviours across salamander populations.

Impact of pesticide applications and land use intensity on enzymatic activity and fitness of reptiles

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Reptiles belong to a group of vertebrates that is globally affected by biodiversity loss. While the underlying causes are highly assorted, environmental pollution (especially in form of pesticides) and habitat loss are recognized as the leading factors for these declines, especially in agricultural landscapes. The reptile order Squamata has thus far been largely neglected whilst studying effects of pesticides on reptiles, whereas the main focus has been given to the orders Testudines and Crocodylia. Overall, reptiles are highly underrepresented in ecotoxicological studies assessing effects of pesticide exposure in vertebrates. Therefore, information regarding potential effects on their organisms, as well as exposure probability and pesticide uptake in the Reptilia has to be considered rather uncharted. Our focus lied in how pesticide applications affect a widely distributed squamate species in Europe. We studied the common wall lizard (*Podarcis muralis*) with regard to enzymatic biomarkers of pesticide exposure (Glutathione-S-Transferase, Glutathione Reductase, Acetylcholinesterase) and body condition. Lizards were sampled from wild populations, along an exposure gradient (three sites with differing land use intensity and one reference site). Our results show a clear impact of pesticide exposure on enzymatic activity of individuals. Body condition of lizards decreased with increasing exposure and gender distribution was skewed in favor to males within exposed populations. Many reptile species regularly come into contact with pesticides and can be expected to suffer from oral and dermal exposure. Thus, we see that it is indispensable for reptiles to be integrated into risk assessments in order to improve conservation practice.

Altitudinal variation in body size and age structure of the Urmia Lake Newt, *Neureergus crocatus* COPE, 1862 in Iraq

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The tendency for organisms to be larger in cooler climates, the so-called Bergmann's rule, has been widely observed in endotherms, but it is debatable in ectothermic animals like amphibians. Altitudinal variations in body size and age of the Urmia Lake Newt, *Neureergus crocatus* were investigated with skeletochronology in 82 specimens (32 males and 49 females) at 5 sites along the altitudinal gradients from 646 to 1626 m in the Northern Iraq. The aim of this study is to determine whether these life history traits vary with altitude and differ between males and females.

The body size in males and females of *N.crocatus* does not follow the Bergmann's or converse Bergmann's cline. The size dimorphism was female-biased and mean body size for females is 15.72 cm, for males 13.59 cm, respectively. The age range is between 6 and 13 years for males and 6 and 15 years for females. There is the significant difference in the age structures between the two sexes, but both sexes do not have higher average ages at higher-altitude sites.

Coming together to conserve a species at the edge of its range: *Triturus cristatus* in the Scottish Highlands

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Conservation genetics has sometimes been criticised for not offering real-world solutions to conservation problems (Pérez-Espona 2017). Our study began as a citizen science project, took a multidisciplinary approach using climate history, ecologists, geneticists and conservation agency staff, and has ended in a habitat management and restoration partnership between farmers, foresters, and government.

The disjunct population of *Triturus cristatus* in the Scottish Highlands was believed to have stemmed from an introduction (Langton & Beckett 1995) until citizen scientists discovered previously unknown populations in relatively inaccessible sites. We evaluated climate and habitat history and postulated a natural means of colonisation c 3000 years BP. Our genetic studies supported this hypothesis and found evidence of at least five separate sub-populations (O'Brien et al 2015).

The isolated nature and its genetic distinctness made this population a conservation priority. We were concerned that its habitat requirements might differ from conspecifics elsewhere and therefore undertook a detailed analysis of 129 environmental variables (Miró et al 2017).

Based on the habitat analysis and our desire to maintain the genetic distinctness of the five putative subpopulations, projects were set up by two government agencies to restore degraded habitat and create new ponds that would be both suitable for breeding and would re-link recently isolated metapopulations. Whilst it is too early to evaluate long-term success, in the first year three of the 25 new or restored ponds have been colonised by *T. cristatus*, and 20 by other amphibians whose larvae form a major component of their diet.

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Loss of genetic diversity prevents adaptation at the margin of distribution in a lentic salamander

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Theoretical studies predicted that if the distribution edge is highly fragmented, the low rate of gene flow may prevent adaptation and range expansion. However, there have been few empirical studies due to the difficulties in identifying the distribution edge of the species. The salamander, *Hynobius tokyoensis*, is endemic to eastern Japan. We have detailed data on the distribution of this species.

In the present study, we tried to clarify the mechanism for determining the distribution limit of the salamander. We analyzed the environmental factors affecting the distribution using an ecological niche models (ENM), and examined the geographical variation of the genetic diversity and morphological traits among local populations all over the distribution area. In the genetic analysis, five microsatellite markers were used and the some genetic diversity indicators, such as heterozygosity, were calculated. As adaptive traits, body size and clutch size were examined for each population. The data of genetic diversity and adaptive traits were sampled from 815 individuals from 46 populations and 716 individuals from 38 populations, respectively.

The ENM showed that the habitats were highly fragmented near the distribution edge. The genetic analyses showed a loss of genetic diversity at the edge of distribution. The body size also decreased at the northern edge of distribution. It was suggested that observed decreasing latitudinal cline in the adaptive traits was the result of maladaptation to cold climates. Therefore, our findings suggested that the habitat fragmentation at the distribution margin prevents the gene flow and adaptation, and therefore restricts range expansion.

Functional variation in the venom of desert vipers *Pseudocerastes* and *Eristicophis* (Viperinae: Viperidae)

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Vipers of the sister genera *Pseudocerastes* and *Eristicophis* represent a poorly studied clade of venomous snake. These ambush predators occupy an array of desert niches, and one species boasts a unique morphological adaptation used for prey capture. The spider-tailed viper, *P. urarachnoides*, has a modified caudal lure which resembles a spider and is used to attract birds. In contrast, the other species of this clade are generalist feeders and incorporate both ectotherms and endotherms into their diet. Little is known about the pathologies induced by these vipers' venoms, though anecdotal reports indicate that envenomation can be severe. The venom composition and medical significance of *P. urarachnoides* are largely unknown, and the antivenom available to treat snakebite from species belonging to this clade is produced using only *P. persicus* venom.

In this study, the venom composition of the four species belonging to this clade were investigated using a combined proteomics (SDS-PAGE, MS/MS) and activity-assay (fluorescence assays, coagulation assays) approach. The neutralising capacity and cross-reactivity of antivenom were also assessed using a suite of immunological (western blotting and ELISAs) and functional assays (using human plasma).

These tests have revealed some substantial interspecific variability in venom bioactivity and composition. In particular, *P. urarachnoides* venom exhibited strongly procoagulant activity which may be linked to its specialised feeding ecology. Despite this interspecific variation, the antivenom demonstrated a high level of cross-reactivity in neutralising the procoagulant toxins of *P. urarachnoides*. This indicates that this antivenom is likely to be an adequate treatment for *Pseudocerastes* and *Eristicophis* snakebite.

Habitat and not heredity shape the thermoregulation of a Mediterranean lizard

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Environmental temperatures and spatial heterogeneity largely affect ectothermic thermal biology (Meiri et al. 2013, Pafilis et al. 2016). Thermal biology may be rigid and determined by heredity (the 'static' view, Hertz et al. 1983) or, alternatively, may respond to directional selection and comply with environmental temperatures (the 'labile' view, Huey 1982). To assess the significance of these two conflicting aspects we focused on three populations of the Erhard's Wall lizard (*Podarcis erhardii*) in the Aegean Sea (Greece). In 2014 we captured individuals of both sexes from Naxos Island (429.8 km²) and released them on two small, lizard-free islets (8 males and 12 females on each one), Galiatsos (0.0073 km²) and Kambana (0.004 km²). We estimated the effectiveness (E), accuracy (d_b) and precision of thermoregulation measuring operative (T_e), preferred (T_{pref}) and body (T_b) temperatures in the three habitats (Hertz et al. 1993). Though T_es were similar, T_{pref} and T_bs differed statistically among the three habitats. As such, the thermal quality (d_e) of the maternal population was found to be lower than that of the two islets. This was reflected in the higher thermoregulatory effectiveness at Naxos, a finding that was further underscored by the differences between d_e and d_b (Blouin & Demers 2001). Within a very short period (three years) lizards shift their thermal preferences and thermoregulatory profile. Since heredity was ruled out, our results advocate the labile view for thermal plasticity.

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Adaptations of the amphibian egg and hatchling for terrestrial nesting in the Wenxian newt (*Tylototriton wenxianensis*)

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Reducing dependence on aquatic habitats for reproduction is a hallmark in vertebrate evolution. Salamanders of the family Salamandridae typically produce aquatic eggs that develop into a free living aquatic larva. Few salamandrid species (genera *Tylototriton* and *Echinotriton*) have adapted to egg laying on land with subsequent aquatic larval development.

We here study the mechanisms that allow successful development of salamandrid eggs on land using the Chinese salamandrid *Tylototriton wenxianensis*. We identified the mechanisms enabling 1) the eggs to develop on land without dehydration 2) hatchlings of *T. wenxianensis* to successfully reach surface water for further development. Water loss and gain rates were compared between eggs of *T. wenxianensis* with those of aquatically reproducing salamanders and agar balls. The contribution of the egg fluid and egg outer membrane to water homeostasis was assessed by replacing egg fluid with water or by removing the outer egg layer. The contribution of egg fluid proteins was assessed using SDS PAGE. The movement of hatchlings towards different potential stimuli was studied in an experimental arena and musculature was examined using histology.

Eggs of *T. wenxianensis* showed high resistance to fluid loss through a combination of the presence of high molecular glycoproteins and retaining a globular shape. Hatching was limited to low light conditions and hatchlings, put on a horizontal surface, actively jumped to a light source using densely organised epaxial muscles, covering distances up to 2.8 m per hour. These adaptations are likely to maximize chances of reproduction success of terrestrial nesting.

Map-like navigational flexibility in a tadpole transporting frog

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The ability to choose direct routes from one familiar location to another that is not perceptible from the first, is a hallmark of a flexible map-like spatial memory structure. Among vertebrates, such ability is being revealed in an ever-increasing diversity of taxa under laboratory conditions. In the field, clear evidence is limited to just a few birds, mammals, and turtles. Despite the diversity of amphibian spatial strategies, very little is known about their spatio-cognitive abilities. Males of a diurnal rainforest frog *Allobates femoralis* transport tadpoles from terrestrial clutches inside their territory to small pools up to 200m away.

To test if *A. femoralis* males can find a direct route to the pools from arbitrary release sites outside their home territory, we translocated tadpole-transporting frogs as they were arriving at artificial pools. We also induced tadpole transport after translocating males from their territories. We tracked the frog movements and manipulated the potential goal-associated cues such as odor by covering, removing, and displacing the pools.

Frogs translocated within their familiar area took direct routes from release sites to pool sites, even if the pools themselves were removed and placed near the release sites. Most frogs translocated across a river and released in an unfamiliar area were not able to locate tadpole depositions sites. Taken together, these results provide strong evidence that *A. femoralis* learn and use map-like spatial representations to find optimal routes over several hundred meters in the understory of the rainforest.

Detection of *Bd* and exploration of microbial communities of amphibians in northern Israel

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Diverse microbial communities reside on the skin of all vertebrate hosts, including amphibians, and for amphibian hosts these communities are considered to play an important role in defence against pathogens. In Israel, little is known about the distribution of the amphibian pathogen, *Batrachochytrium dendrobatidis* (*Bd*), or about the cutaneous microbial communities of amphibians.

Therefore, we investigated the potential presence of *Bd* on amphibians in northern Israel and explored the skin microbial community structure of two co-habiting amphibian species, the rare and only recently rediscovered Hula painted frog (*Latonia nigriventer*) and the abundant and widespread Levant water frog (*Pelophylax bedriagae*).

We documented *Bd* at multiple sites in northern Israel with loads ranging from 0 to 311 zoospore equivalents. These overall low loads could be associated with suboptimal environmental conditions or host-related defences, e.g. the species' mucosome. With respect to host microbiota, we found that the skin bacterial communities of co-occurring Hula painted frogs and Levant water frogs were comprised of mainly Proteobacteria, Bacteroidetes and Firmicutes. However, the community structure differed significantly between host species, suggesting that the skins of these amphibians are unique habitats allowing colonisation of different bacterial taxa. For Hula painted frogs, we also repeatedly sampled individuals across months, finding that the community structure significantly varied through time. These data come together to document *Bd* for the first time and provide a first look at the microbial communities of amphibians in Israel.

Amphibians, roads and evidence-based conservation

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Road networks create exceptionally complex pressures for amphibian populations worldwide and particularly in Europe, where road coverage is among the densest on the planet. However, numerous road-associated threats remain poorly defined for amphibians and unsurprisingly, road mitigation projects are often inadequately monitored and produce unknown population-level conservation improvements. Equally, roads can be used strategically for large-scale species monitoring and prioritisation of landscape-scale connectivity for both meta-populations and habitats but the process needs validation using empirical data and robust implementation. I review our understanding of threats and knowledge gaps for both lethal and non-lethal effects of roads on amphibian populations and discuss monitoring and mitigation solutions using data and examples from multiple recent projects focused on road ecology in the UK. Finally, I discuss conceptual frameworks for mitigation, missing data, common mistakes and potential solutions for improvements of road mitigation projects in relation to the existing evidence-base collated as part of Conservation Evidence at University of Cambridge.

Population traits and diet of the introduced population of the ladder snake (*Rhinechis scalaris*) at Formentera (Balearic Islands, Spain)

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The Pityusic Islands (Balearic Islands, Spain) were meant to be free of any snake species, although during the last decade *Hemorrhoids hipocreppis*, *Rhinechis scalaris* and *Malpolon monspessulanus* were reported in Eivissa and Formentera islands. It has been demonstrated that nursery trade, particularly trade of old olive trees, is the pathway of the introduction of these species since 2003. Ten years later, they were included in the National List of Invasive Species, thus allowing the environmental agency to control the introduced populations.

For the first time, in 2016 the Government of the Balearic Islands carried out a planned fieldwork based on the use of a specially design snake trap to capture as much individuals as possible. 312 *R. scalaris* were captured during 7 months by using 32 to 68 snake traps.

The introduced population showed a sex ratio of 1.00:4.03. Age distribution showed a very structured population, with individuals in all age classes. It highlights that 19.52 % of the captured individuals showed an amputated tail suggesting a similar predation rate than in mainland Spain, where this species comes from, but most of the natural predators are absent in the islands. The digestive analyses showed a great impact on the endemic wall lizard *Podarcis pityusensis formenterae* occurring close to 40.00 % of the digestive content, followed by rodents.

Land-management subsidies for shrub removal are a major threat for herpetofauna in Bulgaria

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Since 2007 Bulgarian farmers have new financial opportunities for land management provided through the Common Agricultural Policy (CAP). Although some CAP and Agri-Environment Schemes (AES) goals are to support biodiversity, their implementation may result in significant loss of habitats suitable for herpetofauna (e.g. 9% decrease of open grasslands' area in four years; Popgeorgiev et al. 2014).

We studied the impact of land management in grasslands on the herpetofauna in three Natura 2000 sites (S and SE Bulgaria) between 2016 and 2017. Twenty-six representative plots with five types of management (from animal grazing to mulching) were selected. Using satellite imagery we evaluated the percent of shrub cover per plot. We found 7 amphibian species (33% of all Bulgarian species, 49 observations) and 15 reptile species (44% of all species, 845 observations). A PCA analysis demonstrated that semi-open grasslands with 20–60% cover of shrub vegetation supported the highest herpetofaunal abundance and diversity. However, CAP subsidies for open pastures currently cause substantial loss of shrub vegetation (especially of important plant communities with *Paliurus spina-christi*) both in the studied areas and nationally. Although we observed extensive direct mortality (e.g. eight *Testudo hermanni* in ca. 2 ha), rapid loss of suitable microhabitats (e.g. for refugia, thermoregulation) is an even greater threat. Major drawbacks of the CAP and AES represent criteria for direct payments inappropriate to the country's natural conditions and a lack of a procedure for assessing the impacts of agricultural activities on biodiversity. Thus, currently CAP and AES in Bulgaria fail in supporting biodiversity and herpetofaunal conservation.

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The effects of background coloration and dark spots on the risk of predation in poison frog models

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Protective coloration is a common predator avoidance strategy in prey animals. Aposematically colored species often display a contrasting color pattern consisting of dark spots of different shapes and sizes on a bright background coloration. Both elements, background coloration and spots are expected to serve different purposes. While the ecological function of the bright coloration has been addressed in many studies, the question of whether the interaction with differently sized spots affects predator behavior has received less attention by researchers.

The study was conducted in a lowland rain forest in Costa Rica. We used 2700 clay models that imitated the polytypic strawberry poison frog (*Oophaga pumilio*) as a proxy for an aposematic prey species. We manipulated the dorsal color pattern by using two aposematic (local and non-local) and a cryptic (non-local) background coloration and combined them with black spots increasing in size (none, small, medium, large). The major objective was to test if spot size alters the survival rate of clay models differing in coloration and origin.

Background coloration and spot size were significant predictors of being attacked whereas the interaction between both effects was not. During five trials the non-local aposematic color morph showed the lowest attack rates and predators did not discriminate between local aposematic and non-local cryptic models. Spot size and attack rate were negatively linear correlated which suggests that predator selection promotes the evolution of dark spots. We further conclude that spot size matters in a contrasting color pattern and plays a crucial role in predator avoidance.

Realized niche and microhabitat selection of the eastern green lizard (*Lacerta viridis*) at the core and periphery of its distribution range

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Range of habitats available and suitable abiotic conditions tend to be narrower towards the periphery of the distribution range of species. Peripheral populations of generalist species could then be more specialized and have a smaller and differentiated realized niche compared to populations at the core.

We compared niche width and microhabitat selection among populations of the eastern green lizard *Lacerta viridis* located either at the core (Bulgaria) or at the northern periphery (Passau, Prague), and estimated niche differentiation among regions. We collected data on vegetation structure and abiotic parameters at the microhabitat scale and built niche hypervolumes to calculate niche size and estimate niche differentiation. Through averaged generalized linear mixed models we analyzed microhabitat differences among regions and microhabitat selection in each region.

Peripheral populations had smaller niche sizes than core ones (Vegetation structure: Plovddiv=90.8sd; Passau=28.8sd; Prague=27sd. Abiotic parameters: Plovddiv=32.8sd; Passau=20.9sd; Prague=23.2sd) and niche differentiation ranged from 60 to 90%. Microhabitats at the periphery had lower radiation and soil compaction, and less structured vegetation. Microhabitat selection at the core depended solely on abiotic parameters, while at the periphery was defined by only vegetation structure (Prague) or a combination of both (Passau). In conclusion peripheral populations of *L. viridis* are more specialized and compensate for overall extreme climatic conditions by responding to different parameters of the microhabitat compared to core populations. Niche differentiation might indicate local adaptation as a possible mechanism for the persistence of populations in the periphery. We suggest specific conservation measures for populations of *L. viridis* in each studied region.

Adult *Bombina bombina* prey on young whitebaits of invasive fish *Perccottus glenii*

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Invasive fish *Perccottus glenii* (*Odontobutidae*) is common in Southern Latvia (Pupina et al. 2015). The need to protect *Bombina bombina* within the territories invaded by *P.glenii* (Pupins & Pupina 2012) makes the study of the possibility of the reciprocal predation between *B.bombina* and *P.glenii* a topical one.

Material and methods. We placed 20 young whitebaits of *P.glenii* (L=10.7±1.34 mm) and 2 adult *B.bombina* in each of 4 experimental aquariums. In control aquariums were only *P.glenii*. Within 6 days, we counted the number of whitebaits in each aquarium. On the third day, after the counting, the amount of *P.glenii* in each experimental aquarium was again adjusted to 20.

Results. *B.bombina* preyed on the whitebaits: the number of whitebaits in the experimental aquariums decreased (1st day - 42.5% of whitebaits were preyed on, 2nd day - 23.75%, 3rd day - 17.5%), in the control aquariums the number of whitebaits remained the same (P <0.05). The predation intensity correlates with whitebaits density (P <0.01). In the second period of the experiment, the effectiveness of predation was higher (84% and 97%) (P <0.05).

Conclusions. Adult *B.bombina* predate young whitebaits of invasive fish *P.glenii*. Such reciprocal predation can be for *B.bombina* both a mechanism for regulating the number of fish predators and competitors, as well for using of a new food resource. The obtained data can be used in the development of measures for protection of *B.bombina* from the invasive *P.glenii*.

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A molecular toxin delivery system in amphibians

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Animals using toxic peptides and proteins for predation or defense typically depend on specialized morphological structures, like fangs, spines or a stinger, for effective intoxication (Mebs, 2002, Nelsen et al. 2014, Fry et al. 2015). Here we show that amphibian poisons instead incorporate their own molecular system for toxin delivery to attacking predators, by using a combination of cellular physiological models and *in vivo* pharmacokinetics experiments. Oral administration of a key toxin of the frog *Xenopus laevis* combined with a naturally cosecreted antimicrobial peptide (AMP), leads to accelerated absorption and higher toxin levels in various organs of live snakes. This enhanced absorption results from the instantaneous damaging of epithelial tissue, involving the formation of intercellular ruptures. Independently evolved AMPs in two other frog lineages enhanced the passage of cosecreted toxins across epithelial barriers in similar ways, confirming a widespread role of these peptides in predator intoxication. Our results drastically alter our perception of how AMPs contribute to an amphibian's survival, and show that toxin delivery systems can range from macroscopic structures down to the pore-forming molecules.

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Genetic structure of the grass snake (*Natrix natrix*) and the midwife toad (*Alytes obstetricans*) in a landscape fragmented by five large-scale transportation infrastructures

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Large-scale Transportation Infrastructures (LTIs; e.g. motorways, railways, etc.) are of major concern because of their high impacts on natural ecosystems and their contribution to habitat fragmentation. Barrier effects of LTIs have been studied on many species, however, most studies focus on single species which limits the understanding of how LTIs affect communities. We studied how five kind of LTIs (a motorway, a main road, a railway, a power line, and a gas pipeline) affect the genetic structure of the snake *natrix natrix* and the amphibian *alytes obstetricans* in the South-West of France. Genetic samples of these two species were collected in 2015 and 2016 on a study site of approximately 250 km². This study site was crossed by the five LTIs arranged in a East-West direction. Genetic samples of 338 *alytes obstetricans* and 130 *Natrix natrix* individuals sampled across the study site were analysed using microsatellite loci. We found a high genetic structure concerning *alytes obstetricans* on the study site but a low genetic structure concerning *natrix natrix*. We will discuss how landscape elements, including the five LTIs, affect the genetic structure of these two species. Dispersal is a key role to prevent small and isolated populations to go extinct. Our results help to better understand which kind of linear man-made structures act as barrier to dispersal concerning two species with high conservation interest.

Tracing the evolutionary rise of an amphibian molecular defence arsenal by resurrecting ancestral skin peptides

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The skin glands of a broad range of frogs (Anura) secrete an arsenal of cytolytic peptides capable to kill pathogenic microorganisms (as part of the immune system) and damage vertebrate epithelial tissues (as an antipredator adaptation). In the family Pipidae, this cytolytic peptide arsenal evolved from a single ancestral hormone gene with an unrelated (nondefensive) function (Roelants et al. 2010, 2013), representing a remarkable case of functional innovation. How and when did cytolytic activity originate in the evolutionary history of this arsenal? And how did this activity change through time to accommodate both immune and antipredator functions? We addressed these questions using a paleogenetic method that involves the phylogenetic estimation, *de novo* synthesis and functional analysis of the peptides produced by the ancestors of pipid frogs millions of years ago. Our results show that cytolytic activity emerged ‘from scratch’, by accelerated mutation of a duplicate hormone gene. This mode of functional innovation is currently considered highly improbable (Bergthorsson et al. 2007), and was recently challenged for the origin of snake toxins (Hargreaves et al. 2014). Interestingly, the earliest peptides showed an antimicrobial activity that surpasses those of most extant peptides. Although activity against vertebrate epithelial cells (supporting the antipredator function) is correlated with antimicrobial activity, some ancestral peptides deviate from this pattern and may be potential templates for antibiotics design. Consequently, our analyses, besides elucidating the rise of a complex amphibian defence adaptation, provide a new way to obtain bioactive molecules with novel structural and functional properties.

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Geographic Variation in adult body length and sexual size dimorphism in the lizard, *Zootoca vivipara*: testing the effects of reproductive mode, lineage, and climate

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Reproductive mode, ancestry, and climatic environment are established determinants of body-size variation in reptiles but their relative importance has rarely been estimated, especially at the intraspecific level (Horváthová et al. 2013, Roitberg et al. 2013, 2015). The lacertid lizard *Zootoca vivipara* occupies almost the entire Northern Eurasia and includes several viviparous and oviparous lineages (Surget-Groba et al. 2006), thus presenting a promising model for such studies. Using original and published data on the snout-vent length (SVL) for more than 10,000 individuals from 72 geographically distinct study samples covering a major part of the species range we analysed how sex-specific adult body size and sexual size dimorphism (SSD) is associated with reproductive mode, lineage identity and several climatic variables. We used general linear models and employed an information-theoretic approach for model selection; potential confounding factors were carefully considered. Populations of the Western Oviparous lineage exhibited a smaller female size and less female-biased SSD than those of the Western Viviparous and the Eastern Viviparous lineages. This pattern persisted

when controlling for climatic variables and is predicted by life-history theory. In contrast, similar differences between the two viviparous lineages became insignificant when tested simultaneously with climatic variables. Remarkably, our best models often included an interaction between lineage and mean winter temperature (a proxy of seasonality) suggesting that not only related species (Ashton 2001, Angilletta et al. 2004) but also intraspecific clades can exhibit disparate body-size clines along similar climatic gradients.

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A new type of aposematism? Flashy feet in Neotropical harlequin toads (*Atelopus*) are a conspicuous signal to predators

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Aposematic visual signals are bright colorations or strong contrasted patterns. In combination with an animal's toxicity, they warn potential predators of its defenses. These signals are either displayed constantly or in the moment of direct threat or attack. The soles of the feet in some populations of the diurnal, toxic Amazonian harlequin toad *Atelopus spumarius* sensu lato (Bufonidae) are bright red and are only seen while walking. In order to be aposematic a signal must be clearly perceivable for predators to learn. To understand how the colorful soles (hereafter referred to as 'red feet') of some *Atelopus* populations are perceived by potential predators and whether they send a stronger signal than populations without red feet, we took spectrometric measurements and calculated contrasts via visual modeling for bird, snake and crab visual systems. The results demonstrate that chromatic information (color) leads to significantly higher conspicuousness of red feet for birds, whereas achromatic information (brightness) makes the red feet also more conspicuous for snakes and crabs. We conclude that red feet found in these toxic toads have an aposematic function as they are clearly visible to potential predators. The red feet are not a continuous warning color display, neither are they part of any deimatic display. We therefore suggest them to represent a so far unrecognized type of aposematism. The bright red feet are only visible when the toad is walking thus, the feet are 'flashing'. We suggest the term 'flash mark aposematism'.

Environmental stress as an endocrine disrupter in tadpoles of *Xenopus laevis* and *Rana temporaria*

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Changes in biotic and abiotic factors may alter or disturb the endocrine system such as the thyroid hormone system in larval amphibians. Therefore they crucially determine larval development, growth, and regulation of energy metabolism, especially until the onset of metamorphosis. In addition to energy stored in the tail, tadpoles store energy in the form of fat in the liver which is required for metamorphic transformations. We studied the endocrine disruptive effects of environmental stress by altered thyroid hormone concentrations on the larval growth, development and body condition of two frog species with different post metamorphic life histories, the Common frog *Rana temporaria* and the African clawed frog *Xenopus laevis* at the onset of metamorphosis. We used the hepatosomatic index (HSI) and Fulton's condition factor (FCF) as body condition indices. To simulate environmental stress which increases thyroid hormone concentration tadpoles were treated with L-Thyroxin. For a decrease of thyroid hormone concentration tadpoles were treated and with sodium perchlorate, an environmental relevant toxin. We show that endocrine disruption due to environmental stress leads to significant effects on body condition in metamorphic tadpoles in both species. Whereas in *Rana temporaria* only FCF was influenced, both condition indices in *Xenopus laevis* were influenced by environmental stress. Our results show the effect of environmental stress on body condition, development, and growth in both species although to different extent. This is crucial since a lower body condition at the onset of metamorphosis is known to influence body condition and thus, individual fitness in later life stages.

The use of clay models to assess potential predation on cave salamanders

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Clay models are frequently used in ecological studies to estimate potential predation rates on small and cryptic vertebrates (Bateman et al. 2016). These models are easy to shape and they retain predator marks, allowing the identification of the body part attacked and of the predator. In herpetological studies, prey models are used to evaluate colour polymorphism, aposematism, mimicry and to assess differential predation rates in different habitats. Clay models have been applied in studies on both tailed and tailless amphibians in different geographic regions, with a first application in Europe by Velo-Antón & Cordero Rivera (2011).

The presented research aimed to evaluate, by means of realistic clay models: i) if different potential predation rates were observed on the plethodontid salamander *Speleomantes strinatii* living in different habitats, and ii) if the presence of a brooding female reduced the potential predation of attended egg clutches, in comparison to unattended clutches.

The results show that predators attacked the models significantly more ($P < 0.01$) in woodlands (43 attacked models out of 90) than inside caves (16 attacked model out of 94). Therefore, salamander populations inhabiting underground environments may benefit from reduced predation risk in comparison to forest-dwelling populations (Salvidio et al. 2017). Concerning the second experiment, similar predation rates on isolated eggs and on eggs attended by females were observed. This may suggest that all kind of models (i.e. eggs and/or salamanders) were not discriminated by the predators and attacked with similar frequencies.

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Development of the fire salamander: morphology and gene expression similarities to anurans

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Characterizing the development of the Fire salamander, *Salamandra salamandra*, is paramount to study its ecology and evolution. Because metamorphic changes in caudates are less pronounced than in anurans, it was uncertain if similar post-embryonic main stages are present. We analyzed the morphological change, gene expression and alkaloid profiles in fire salamander larval development to categorize it into main stages and explore the developmental origin of the metabolism of salamander alkaloids. For this, we reared larvae under controlled conditions and sampled them at fixed time points. Three distinct clusters (PreM, ProM and Meta) were obtained from the morphological analysis of 46 larvae, and confirmed by the microarray-derived gene expression profiles of 23 of these larvae, further splitting ProM into early and late ProM. A total of 3510 probes targeted transcripts differentially expressed between these clusters.

Genes related to organogenesis were up-regulated in PreM, and structural proteins and genes related to anatomical structure development and pigmentation, including the biosynthesis pathways of pigments like pteridines and melanin, were up-regulated in late ProM and Meta. The alkaloid profiles of 19 larvae, based on gas chromatography, tracked the developmental origin of steroidal alkaloids biosynthesis to ProM. Comparing fire salamanders to two anuran species, we found molecular similarities in basic metamorphic processes, like skin restructuring and tail fins resorption. This flagged the hypothesis that the herein characterized postembryonic developmental main stages, PreM, ProM and Meta, might be homologous to the respective main stages known for anurans, premetamorphosis, prometamorphosis and metamorphic climax.

Applying integrative demography to obtain reliable effective/census size ratios in pond-breeding amphibians

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The ratio of the effective number of breeders (N_b) to the adult census size (N_a) provides relevant information for assessing population status and understanding evolutionary processes operating at local scales, but estimating both parameters is challenging. We argue that integrating the sibship frequency (SF) method and capture-mark-recapture (CMR) robust design models is an optimum means of estimating the N_b/N_a ratio in seasonal-breeding species.

We illustrate this approach using genotypes of three pond-breeding amphibian species (*Epidalea calamita*, *Hyla molleri* and *Pelophylax perezi*, $n = 73$ -96 single-cohort tadpoles and 94-300 adult individuals per species genotyped at 15-17 microsatellite loci) to estimate N_b in a locality in Central Spain. We then assess the reliability of N_b estimates by comparing sibship inferences with field-based information and check for the convergence of results in replicated subsampled analyses. Finally, we use CMR data from a 6-year monitoring program to estimate annual N_a for the three species and calculate the N_b/N_a ratio.

Reliable N_b/N_a ratios were obtained for *E. calamita* ($N_b/N_a = 0.18$ -0.28) and *P. perezi* (0.5). On the other hand, in the case of *H. molleri*, N_a could not be appropriately estimated and genetic information proved insufficient for reliable N_b estimation. Our results show that integrative demographic studies taking full advantage of SF and CMR methods can provide accurate estimates of the N_b/N_a ratio in seasonal-breeding species, and also allow for ready evaluation of the reliability of results. This represents a good opportunity for obtaining robust demographic inferences with wide applications in evolutionary and conservation-oriented research.

Ontogeny and differential substrate use among populations of the spiny footed lizard (*Acanthodactylus schreiberi*): Plasticity or an ongoing evolutionary adjustment?

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Sprint performance is crucial for success rates of lizards in foraging, intra-specific interactions and survival (McElroy et al. 2008). Even though physiological and behavioral strategies determine performance levels among taxa, substrate can also “shape” morphology (hence physiology) and/or behavioral sprinting modes (Vanhooydonck et al. 2015).

In the current study, we used adult individuals of *Acanthodactylus schreiberi* from different habitats (sub-urban shrubland, pine forest, coastal sand dunes), and estimated their sprint performance (maximum speed and maximum instant acceleration) and substrate use on different types (simulated dirt, rocky and sandy substrates), using a custom racetrack.

Geri (site where dirt prevails but rocks and graded soil are also present) individuals appeared to be the most generalist performers (in terms of performance levels and substrate use), as their habitat consists of three substrate types. Sprint performance and substrate use for populations from Agros sites (rocky substrate) and Akrotiri (sandy substrate) can be explained by their respective habitat/substrate types. Agros individuals tend to use forelimbs at a higher degree while running, presumably because they are used to live in habitats with many obstacles, while individuals from Akrotiri relied mostly on their hind limbs. This is in accordance with previous studies reporting that taxa living in open habitats extensively use their proportionally longer hindlimbs (a finding also evident among the populations studied herein), while running (Herrel et al. 2002). These results can increase our understanding of locomotion mechanics and shed light onto the role of habitat in differentiating morphology and behavior, even among conspecific populations.

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Informed conservation management on yellow-bellied toads: influence of different land use and succession on population dynamics at a former military area

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The yellow-bellied toad (*Bombina variegata*) is one of the most endangered amphibian species in Germany. Populations and ranges are declining for years due to habitat loss (Gollmann & Gollmann 2002). Quantitatively identifying local stock situation, dynamics and their causes is essential to implement effective conservation measures on isolated populations. This exemplary assessment of different management forms influencing *B. variegata* populations on a former military area started in 2016 and will serve as a model study to improve supra-regional conservation.

Population dynamics and demography under different land use and succession, which potentially affect isolation and dynamics of local populations, have been analysed in four study subareas: pioneer site, extensive clay pit, semi-open pasture landscape and abandonment (former pioneer site).

There is a significant demographic difference (one-way-ANOVA; $p = 0.005$) between subareas indicating isolation of populations. Migration distances seem to be lower than those stated by Herrmann (1996) and Gollmann et al. (2000). Comparisons to previous studies on one of the subareas (Cullmann 2008, Grünewald-Buschmann 2008, Grünig 2005, Sweatt 2005, Ulmen 2006) show a negative demographic trend from 2004 to 2016, maximum life expectancy halved (two-way ANOVA; $p = 0.0014$). Body condition determined by residual index (Băncilă et al. 2010) improved (one-way ANOVA; males: $p = 0.0033$; females: $p < 0.001$), indicating higher food availability (Scheele et al. 2014) due to increasing succession. Estimated population size basing on mark-recapture analyses (Chapman estimator) varies interannually and recovered quickly from decline after reducing succession. Ongoing research will indicate whether life expectancy of individuals in reference areas is low as well, requiring adapted conservation management.

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Assisted colonization of juvenile Western Swamp tortoise *Pseudemydura umbrina* into novel wetlands: macro invertebrate communities, biomass and turtle diet

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The seasonal swamp habitat of the critically endangered Western Swamp Turtle (*Pseudemydura umbrina*, SIEBENROCK 1901) is confined to a small area north of Perth, Western Australia. Rainfall in this area declined over the last five decades. This trend is predicted to continue, increasingly shortening the annual activity period for the turtles and potentially shifting their optimal climate zone a few hundred kilometres to the south. Since habitat loss and fragmentation impede any natural movement of *P. umbrina* populations we tested in a 12 month trial the feasibility of their assisted colonization into a wetter, but also cooler climate to the south coast of Western Australia where conditions may become optimal for the species in 30 to 60 years.

Thirty six captive-bred juveniles >100 g body mass from Perth Zoo were released into three novel wetlands, two about 300 km south of Perth and a reference one north of Perth. Fifty-four samples of macroinvertebrates were taken at those three release locations to provide information about invertebrate communities and food availability. Sampling time was adjusted according to weather conditions, water levels and the seasonal drying of swamps. Both invertebrate biomass and biodiversity was lower at the southern sites with cooler climatic conditions. Food choice and diet was investigated by flushing once the stomach of each turtle. Preliminary results indicate that the southern areas may be suitable future habitats for *P. umbrina*. Assisted colonization into novel wetlands may become a reasonable conservation tool to ensure the long term survival of the species.

Data fabrication by Paul Kammerer: the testimony of Franz Megusar

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Paul Kammerer (1880-1926) was a highly controversial biologist, publishing on the inheritance of acquired characters, mostly in amphibians (*Alytes*, *Proteus*, *Salamandra*). After his suicide, following discovery of manipulation with the only remaining experimental specimen, he was widely regarded as a forger, but Koestler (1971) promoted the idea that Kammerer might have been the victim of an intrigue. Recently, his work has been discussed in the context of epigenetic inheritance (Vargas et al. 2016), whereas van Alphen & Arntzen (2016) concluded that Kammerer had presented animals acquired from nature as outcome of experiments.

Franz Megusar (1876-1916) was Kammerer's colleague at the Biologische Versuchsanstalt in Vienna. In 1913, he voiced severe criticism of Kammerer's methods and conclusions at an important congress.

In the archive of the Natural History Museum Vienna we recently discovered manuscripts by Franz Megusar, pertaining to the controversy about Kammerer's work. We present some of Megusar's comments and observations on Kammerer's research and his own attempts to replicate experiments on colour change in salamanders.

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Hidden treasures: old museum tadpole collections in the light of modern scientific research

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Due to their free-living larvae, anurans are unique among all terrestrial vertebrates. The larvae present a distinctly different morphology compared to their adult stages and exhibit a huge diversity of morphological characters, which have proven to be remarkably plastic in response to ecological factors in some species. Tadpoles are therefore of particular scientific interest as they can provide key information for the understanding of species evolution, ecology and conservation. However, amphibian research still primarily aims at adults and information on tadpoles is often scarce. In scientific (museum) collections, tadpoles are often disregarded and barely processed and thus are 'hidden treasures' for scientific research. The phylogenetic and geographic distribution of tadpoles' morphological diversity is still barely understood. The underlying question, what determines the tadpole's morphology – shared evolutionary history or ecological factors – remains largely unanswered.

The aim of this ongoing study is to contribute to the identification of phylogenetic and geographic patterns in tadpole morphology. Tadpole specimens collected in different regions of the globe and housed in museum collections in Germany and Switzerland are considered. The available data from these collections will be used to build a tadpole database which, combined with available phylogenetic and ecological data, will contribute to the understanding of patterns in tadpole morphology.

To achieve these aims, 'classical' museum collection work and modern morphological, molecular, statistical and GIS methodology are combined. This interdisciplinary project therefore unlocks inactive museum collections and provides a comprehensive analysis and dataset of tadpole morphology in the light of species' phylogenetic and geographic distribution.

Evolutionary Cytogenetics of Proteid Salamanders

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The salamander Family Proteidae includes only two genera, the North American *Necturus* and the European *Proteus*, whose phylogenetic relationships have long been problematic. Cytogenetic evidence underlines the uniqueness of Proteidae among urodeles (Sessions, 2008). Proteids share a chromosome number ($n = 19$) and other karyological features that are not found in any other salamander group. Also, both genera are characterized by relatively large genomes and *Necturus* includes the largest genome size reported for any tetrapod (Gregory, 2005). *Necturus* (five species) is characterized by strongly differentiated XY sex chromosomes with interspecific differences that may be significant phylogenetically as well as in sex chromosome evolution (Sessions and Wiley, 1985). *Proteus* (one species) has a unique sex chromosome system, apparently derived from the *Necturus* heteromorphism, involving an ancient X-Y translocation that has become fixed as a homologous pair of XY chimeric chromosomes in both males and females (Sessions et al., 2016). The meiotic behavior of the X and Y sex chromosomes in *Necturus*, shown here for the first time, provides clues to how this translocation occurred in the ancestor of *Proteus*. The predicted and observed reproductive consequences of the XY translocation suggest that it has played an important role in *Proteus* evolution.

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Who is who in *Gloydius* Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, EE51014 Tartu, Estoniarmedius complex? New insights from extended geographical sampling and consequences for venom biochemistry research

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The pit-vipers of *Gloydius halys-intermedius* complex are a group of medically important venomous snakes widespread in Asia. The biochemistry of their venom components were extensively studied during the last decades. However, this species complex has been remarkable by very complicated taxonomy till recent time. It raised a lot of confusion, especially in biomedical and biochemical publications. We reviewed taxonomy, systematics and distribution of the members of the complex, based on our original and GenBank data on variability of mtDNA markers. Geographically broad sampling was achieved, including the type localities. The final dataset comprised of 186 individuals.

The following species should be recognized based on combined genetic and morphological evidences. *G. caraganus*: steppe and semi-desert zones of Kazakhstan and Uzbekistan, including northern areas of Kyrgyzstan and Turkmenistan; *G. caucasicus*: Talysh, Elburz, and Kopet-Dag mountains; *G. changdaoensis*: Changshan Islands and adjacent mainland; *G. cognatus*: from east Kyrgyzstan and Kazakhstan to central and northeast China, including Mongolia and Russia (Republics of Altai and Tuva); *G. halys*: South Siberia (Russia), part of northeast Mongolia and northeast China; *G. intermedius*: Russian Far East, Korean peninsula, and northeast China; *G. rickmersi*: known from Alai valley only (Kyrgyzstan); *G. shedaoensis*: Shedao Island and adjacent mainland; *G. stejnegery*: China (Beijing, Liaoning, Shaanxi and Shanxi provinces). *G. lijianlii* must be synonymised with *G. changdaoensis*, while *G. saxatilis* is synonym of *G. intermedius*. It is highly recommended to use molecular markers to assist morphological identification of specimens from the territories potentially inhabited by multiple species of this diverse group.

Brushwood removal from ditch banks attracts breeding frogs in drained forests

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Forestry drainage has transformed extensive North-European wetlands to homogeneous forests with a dense network of open ditches. In such drained wetlands, small sun-exposed temporary water-bodies – a favoured breeding habitat for amphibians – are replaced by linear ditch corridors that become increasingly shaded by woody vegetation.

We explored whether simple removal of woody vegetation from ditch corridors can increase the habitat quality for two frog species, *Rana arvalis* and *R. temporaria*, in a drained pine-wetland landscape in Estonia. Such practice is compatible with the forestry purposes of maintaining the ditches and access to the area. In a before–after–control–impact (BACI) experimental design, the mean shade above cleaned ditches decreased from 66% to 35% and appeared to be the main reason for a dramatic increase in frog breeding in the next spring. The change increased numbers of both species and was based on colonization of the ditches from the surrounding landscape.

We suggest that keeping forest ditches exposed to the sun may mitigate the overall negative drainage impact on wetland species at least in the short term, and brown frogs could be used as focal species to guide these practices. In the longer term, however, such systems may not be viable without stable source populations in protected or restored wetland patches.

The long-term cost of chytridiomycosis for yellow-bellied toads

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The chytrid fungus *Batrachochytrium dendrobatidis* [Bd] is globally renowned for its devastating impact on amphibian populations. However, even lethal pathogens may not, under some circumstances, cause significant illness and mortality in susceptible hosts.

We studied two native populations of yellow-bellied toads (*Bombina variegata*) with fluctuating Bd levels under natural conditions over a five-year period. Using the unique belly patterns we were able to distinguish between individuals throughout this period. We used Bayesian statistics for studying the long-term coexistence of the populations of this sensitive species with Bd.

We look in the Bd infection dynamics where we regard the high, but fluctuating, prevalence of Bd, the environmental conditions, the impact of body condition, recruitment and survival on population persistence and quantify the cost of endemism.

Phylogeography and population genetics of the angulate tortoise *Chersina angulata*

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Chersina angulata is a medium-sized tortoise endemic to southern Africa, occurring from southwestern Namibia along the coastline to the Eastern Cape Province in South Africa (Hofmeyr 2009). Former studies based on mitochondrial markers raised the question of whether two distinct species exist within *C. angulata* that diverged in the late Miocene (Daniels et al. 2007).

Our study aims to get a clearer picture of the genetic structuring using additional 14 microsatellites to examine whether gene flow exists between the clades. In total, we analyzed 240 samples from 68 populations.

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Do albino snakes experience higher rates of attack from avian predators than wild-type snakes? – An experimental test using plasticine models

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Albinism is a distinctive and usually rare variant of body coloration arising from the absence of integumentary melanin, and linked to a variety of skin and ocular disorders. Albinos are usually assumed to exhibit higher mortality compared to non-albinos; however, direct tests of this hypothesis are lacking. As birds exhibit acute color vision and may serve as strong selective agents on prey coloration, we asked whether albino snakes were subject to different rates of attack (and thus mortality) than wild-type forms as a function of bird predation.

We constructed 800 plasticine snakes that mimicked the form and coloration of wild-type and albino eastern gartersnakes (*Thamnophis s. sirtalis*), as well as two mixed color treatments that represented intermediate forms. Models were placed in the field at a locality where both eastern gartersnakes and potential avian predators are present in 2015 ($N=200$ models; 50 models/treatment) and 2016 ($N=600$ models; 150 models/treatment), then collected and scored for evidence of bird attack.

Avian attack rates across all model groups (10%) were consistent with those reported from similar studies, suggesting that models served as reasonable snake proxies. However, we found no difference in attack rates across treatments, either within years (2015: $P=0.33$; 2016: $P=0.63$) or across both years combined ($P=0.32$).

We suggest that the rarity of albinos in eastern gartersnakes and perhaps other terrestrial vertebrates may be due less to predation arising from increased conspicuousness to predators than other potential correlates of albinism, such as compromised thermoregulation or elevated rates of skin disease.

The Amur rat snake (*Elaphe schrenckii*) in the Netherlands. Distribution, habitat use and reproduction

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In 1994 multiple Amur rat snakes (*Elaphe schrenckii*) were released in Eelde (Drenthe province, the Netherlands). After the apparent establishment of a population, speculations on the species' status have been topic of discussion ever since. However, no (scientific) research had ever been conducted prior to 2016. Since *E. schrenckii* is an alien species in the Netherlands it should be monitored closely.

Therefore a study was carried out between April and November of 2016. Citizen science was used to determine the species current range. A total of 1496 interviews were conducted in 111 square kilometer grids surrounding the initial release site. To determine home range and habitat use, seven specimens were radio tracked five days per week.

The species has been recorded in 10 1x1 km square grids. 97.5% of all observations occurred within 1 km distance from the initial release site and 59.1% within a 250 meter radius. Home ranges (MCP) varied between 3.5 and 19.9 hectares ($\bar{x}=11,02 \pm 7,51$). Minimum total distances travelled varied between 2.471 and 4.447 meter. Maximum displacement within 48 hours is 649 meter. Preferred habitats comprised mostly of well-structured, sun-exposed vegetation at the edge of open fields and gardens but *E. schrenckii* presence has also been recorded in gutters and on rooftops. Interestingly, compost heaps seem to be appealing and specimens can spend weeks on end in or nearby these structures, especially during the oviposition period. Indeed these heaps are utilized for oviposition as was proved by the discovery of eggs and hatchlings within these structures.

Phylogeny and diversification of mountain vipers (*Montivipera*, NILSON et al. 2001) in the Near and Middle East

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The Near and Middle East are hotspots of biodiversity, but the region remains underexplored at the level of genetic biodiversity. Here we present an extensive molecular phylogeny of the viperid snake genus *Montivipera*, including all known taxa.

Based on nuclear and mitochondrial data we present novel insights into the phylogeny of the genus and review the status of its constituent species.

Our analyses revealed a montane origin of *Montivipera* at 12.3 Mya and support substantial changes in effective population size through Plio-Pleistocene periods. We conclude that climatic oscillations were drivers of allopatric speciation, and that mountain systems of the Near and Middle East have strongly influenced the evolution and survival of taxa, because climatic and topographical heterogeneities induced by mountains have played a crucial role as filters for dispersal and as multiple refugia. The wide diversity of montane microhabitats enabled mountain vipers to retain their ecological niche during climatic pessima.

In consequence the varied geological and topographical conditions between refugia favoured genetic isolation and created patterns of species richness resulting in the formation of neoendemic taxa. Our data support high concordance between geographic distributions of *Montivipera* haplotypes with putative plant refugia.

Morphometric characteristics of alpine salamanders; a support for subtaxonomy and conservation?

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Within alpine salamanders, Mikšić (1969) described the subspecies *Salamandra atra prenjensis*, isolated in the Dinarides from its nominal (subspecies) population (*Salamandra a. atra*) occurring throughout the Alps. Although Dinaric populations group in a separate phylogenetic clade (Helfer, 2010), their subspecies status has been under debate (e.g. Klewen, 1988). The official taxonomic name has corollaries for conservation and management (Zachos, 2016) which is the primary reason of importance for solving species subtaxonomy.

We tried to assess morphological characters diversifying the Alpine from Dinaric population by measuring 14 traits of 62 alpine salamanders from Austria, and 189 from Bosnia and Herzegovina. Within each country, two allopatric subpopulations were measured.

Results show that the pattern of trait correlation differs between the two populations, and that the two differ on 78% of observed traits, and group in two respective clusters. Dinaric individuals are smaller and have less costal grooves compared to their Austrian relatives. Besides having a swollen cloaca, males have bigger limbs and heads, while females are bigger and more robust. Sexual dimorphism is more pronounced within the Austrian population.

Results are discussed in relation to the habitats, as Dinaric individuals mostly inhabit areas above tree line, whereas most of Alpine populations are found in forested areas. A registered positive correlation between geographic distance and morphology, suggests that the morphological differences might increase with time due to both: geographical isolation and habitat differences. Results support that *ssp. prenjensis* should be accepted as separate subspecies taxon and that these populations deserve an individual conservation approach.

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New distributional records for the herpetofauna in the southern Ethiopian Highlands

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New records that extend ranges of 3 amphibian and 3 snake species are reported on the basis of specimens collected in the southern part of the Harena Forest (Bale zone, Oromia Province, Ethiopia), at elevation 1500–1600 m a.s.l.

In a series of *Phrynobatrachus* Günther, 1862, individuals of two phenotypes are present that differ from each other and from frogs of this genus previously collected or photographed at other localities in this country. Some of their traits resemble *Ph. minutus* (Boulenger, 1895), *Ph. inexpectatus* Largen, 2001, but also *Ph. scheffleri* (Nieden, 1911) – a species that is not known from Ethiopia so far (Largen, 2001; Largen & Spawls, 2010). Also, if these specimens do belong to *Ph. minutus* and *Ph. inexpectatus*, this would be another locality where they occur syntopically.

A record of *Tomopterna kachowskii* (Nikolski, 1900) adds a new taxon to the list of amphibians of the Harena Forest (Largen & Spawls, 2011). Moreover, this frog that is believed to be a savanna species was now found in an unusual habitat – a tall primary forest.

A new record extends the range of *Pseudoboodon gascae* Peracca, 1897 with a locality that is separated by around 300 km from the nearest of the previously known. This is the tenth specimen of this snake ever collected and the first found in a forest.

Two other snake species that I report for the first time from this locality are *Afrotyphlops blanfordii* (Boulenger, 1889) and *Crotaphopeltis hotamboeia* (Laurenti, 1768).

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Reptiles from the Tropical Andes: A decade of wonderful discoveries

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Despite extensive progress in species description and documentation during recent decades, the discovery and analysis of biodiversity in hotspots, such as the Tropical Andes and Mesoamerica, is still a work in progress. Reptiles from South America are among the least known groups of vertebrates. Nearly 100 new species of reptiles, mostly from the Andes, have been described from Ecuador and Peru in the 21st century alone.

Since 2009, the Zoology Museum at Pontificia Universidad Católica del Ecuador (QCAZ) has been involved in a long-term research project that aims to document the biodiversity of Ecuador. To achieve this, we have performed intensive sampling of poorly explored areas along the Andean cordillera. Based on morphological evidence and phylogenetic analyses of DNA sequence data, we have discovered and described 25 new species of reptiles from the Andes of Ecuador and Peru currently allocated in the following genera: *Alopoglossus* (1), *Anolis* (3), *Atractus* (2), *Enyalioides* (6), *Imantodes* (1), *Pholidobolus* (2), *Phyllodactylus* (1), *Riama* (3), *Stenocercus* (1), *Synopsis* (4), and *Trilepida* (1).

We have also recorded the lizards *Anolis heterodermus*, *Macropholidus ruthveni*, and the snakes *Atractus typhon*, *Coniophanes longinquus*, and *Tantilla alticola* for the first time in Ecuador.

In addition, we have studied the phylogeny and biogeography of a few clades, with a strong focus on Cercosaurinae, the most diverse clade of South American gymnophthalmid lizards, for which we have proposed several taxonomic changes based on phylogenetic evidence. Our data indicate that many other species of reptiles from the Tropical Andes await discovery.

A leap forward in the conservation genetics of *Proteus* populations

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The olm, or proteus, (*Proteus anguinus*) is arguably the most enigmatic taxon of Europe's herpetofauna. Proteus research has still to catch up with the recent advances in other European amphibians, such as the molecular taxonomic revolution and conservation biology. Objectively, advancement is hampered in three ways. (1) The subterranean habitat of proteus is extremely difficult to access; a large fraction will always remain concealed. (2) Proteus research requires its own special methodology that has yet to be developed and tested. (3) As populations cannot be censused by conventional methods, no reliable data on population trends are available, and their formal status is purely speculative. This seems unacceptable for an Annex II priority species of the EU Habitat Directive, but reflects the current state of knowledge.

This contribution is an overview of our latest progress on the phylogeographic structure, nuclear marker development, genetic diversity and population size assessment of proteus. The entire taxon is deeply subdivided into seven to nine mitochondrial DNA lineages that are corroborated by nuclear DNA. We applied a set of newly developed tetranucleotide microsatellites to further assess the structure and diversity within lineages. Populations from hydrologically interconnected caves seem to share migrants at high rates, which is good news from a conservation perspective. Genetically marked individuals were re-caught in order to estimate local population size by mark-recapture modelling. First results suggest that a proportion of the population is highly territorial, while other animals migrate to or from hidden sections of the subterranean karstic aquifer.

Investigating the effects of infection with *Batrachochytrium dendrobatidis* on life history traits and skin toxin production of *Bufo bufo* tadpoles

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Batrachochytrium dendrobatidis (*Bd*) is the causative agent of chytridiomycosis, a major cause of worldwide amphibian biodiversity loss. The pathogen is known to be present in the Carpathian Basin (CB), but dramatic declines of amphibian populations have so far not been reported for this region. The lack of catastrophic effects may be due to low pathogenicity of the local *Bd* strain and/or high tolerance of local amphibian populations.

Here, we experimentally investigated direct and indirect effects of larval exposure to two concentrations of *Bd* zoospores (~20 or ~2000 zsp/ml) on life history traits and skin toxin production during early ontogeny of common toads (*Bufo bufo*). We used the *Bd*-GPL strain, which is not present in natural populations in CB, but has a worldwide distribution and is highly virulent.

We observed relatively high infection intensities (up to 5000 GE/ml) at the high zoospore concentration, but no significant effect on survival, body mass or development rate either in larvae or during metamorphosis. Nonetheless, exposure to high *Bd* zoospore concentrations resulted in lower skin toxin content of toadlets 14 days after metamorphosis compared to controls. It appears that common toads in CB are tolerant to *Bd* infection, at least during early life stages, but negative effects of infection may nonetheless appear, such as in the form of lowered skin toxin production.

Different (sub)species or not different (sub)species? - study of the contact zone between two taxa of *Vipera berus* and *Natrix natrix* in Switzerland

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Speciation is a complex process and classifying taxa currently in speciation in “delimited” boxes is even more difficult. In order to determine the specific status, we analysed the contact zones between two snakes species that are present in Switzerland with at least two subspecies or/and distinct genetic lineages: *Vipera berus* with the Northern and Italian/Alpine mtDNA lineages and *Natrix natrix* with the two subspecies *N. n. natrix* and *N. n. helvetica*). For both nuclear and mitochondrial genomes were analysed, as well as morphological parameters.

Contrasted results have been found between the two species: For *Natrix natrix*, a lack of gene flow was found at the contact zone, even between individuals geographically separated by a few kilometres. Morphological differences were also present even a single parameter cannot differentiate individuals with high confidence. On the opposite, a large gene flow has been observed between mitochondrial lineages in *Vipera berus* even if this species is not considered as highly mobile. Statistical differences between several morphological parameters were found, but correct classification using discriminant analyses was very limited, suggesting that the two lineages are morphologically very similar.

Consequently, this study is an additional example of the difficulty to determine what is a good species, but also highlights the importance of studying contact zones to evaluate the specific status of taxa.

Competition induces increased toxin synthesis in tadpoles of the common toad (*Bufo bufo*)

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Chemical defences are widespread in animals, and are important in shaping biotic interactions such as predation and pathogenesis. The role of defensive chemicals in deterring competitors through allelopathy is demonstrated in lower, sessile animals, but little is known about its significance in phylogenetically higher, mobile taxa. Our previous studies suggested that bufadienolide toxin secretion in common toad (*Bufo bufo*) tadpoles may be elevated by competition for food. Here we provide experimental evidence to confirm this hypothesis.

We conducted a microcosm experiment in which we manipulated conspecific and heterospecific competitor densities, using larvae of the agile frog (*Rana dalmatina*) as heterospecifics, and subsequently analysed bufadienolide content of toad tadpoles using HPLC-DAD-MS. We investigated allelopathic effects of toad larvae on agile frog tadpoles by measuring their body mass and estimating their developmental rate.

We found that total bufadienolide content of toad tadpoles was positively related to competitor density, demonstrating competition-induced plasticity in toxin production. However, heterospecific competitors did not have a stronger effect than conspecifics, and there was no significant difference in body mass and developmental stage of agile frog larvae between treatments. Our results thus do not support an allelopathic function of bufadienolides. Instead, they suggest that inducible changes in toxin production of toad tadpoles may have evolved to mitigate risks posed by competitors, including aggression, cannibalism or disease.

Gene flow and unexpected ancient divergences in Western Palearctic pond turtles (*Emys* spp.)

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Using range-wide sampling, we analyse genetic differentiation and gene flow patterns in pond turtles (*Emys* spp.), with a special focus on secondary contact zones.

Based on population genetic analyses of highly polymorphic microsatellite and mitochondrial markers, we show that genetic differentiation matches well with general Western Palearctic distribution patterns and current taxon delimitation. However, individual contact zones and introgression patterns differ across the distribution range, with limited gene flow between some taxa and broad-scale gene flow between others.

Although the distribution pattern suggests that the individual mitochondrial lineages dispersed from former glacial refugia, or are still largely confined to their refuges, fossil-calibrated molecular clock calculations reveal Miocene and Pliocene divergence ages.

Our long-term investigations in the genetic differentiation of European pond turtles result in an excellent understanding of the biogeography of a wide-ranging animal species distributed across a major part of the Western Palaeartic.

The changing views on the evolutionary relationships of extant Salamandridae (Amphibia: Urodela)

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The Salamandridae are the second speciose family of tailed amphibians. Currently, 21 genera with 117 species are recognized within three sub-families. The family has a Holarctic distribution, with the majority of species living in Europe and Asia. Apart from their morphological and ecological variability, the Salamandridae are known for their exceptionally broad spectrum of reproductive strategies and breeding behaviours. We here review the changing views on the evolutionary relationships of extant salamandride genera over the last 90 years. We transfer published phylogenies into simplified cladograms to enhance comparison. Extinct taxa are omitted, so classical morphological trees, which often include paleontological data, can be easier compared to molecular phylogenies that have to rely on existing taxa only. Since the 1990, molecular phylogenies changed our view on the relationships of genera, interestingly with some remarkable divergence among different molecular trees. These incongruencies were caused by preconceived opinions about monophyletic groups of taxa as well as the selection of characters and genes analysed.

Title: Understanding hybrid zones between divergent phenotypic and genetic populations of fire salamanders

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The evolution of distinct phenotypic groups that can coexist in sympatry is a major topic in evolutionary biology. Hybrid zones, which are generally formed after secondary contact between species or lineages, are crucial to understand the evolution of phenotypic traits and introgression patterns. The fire salamander, *Salamandra salamandra*, shows remarkable intraspecific variation in phylogeographic structure and phenotypes (body size and colour pattern), but more strikingly, in reproductive modes. Northern Iberian populations of fire salamanders constitute an exceptional natural system to study the evolution of phenotypic forms, where two reproductive modes and up to four subspecies occur.

Here we present an overview of the distribution of main lineages in *S. salamandra*, identify their contact zones and analyse hybrid zones between divergent phenotypic and genetic lineages across Northern Iberian populations. In particular, we investigate gene flow and patterns of introgression between *S. s. bernardezi* and 1) *S. s. gallica* in northern Galicia, 2) *S. s. bejarae* in the Cantabrian Mountains and 3) *S. s. fastuosa* in the Asturias-Cantabria border. We integrated fine-scale sampling (ca. 100 samples per contact zone), genetic information (mitochondrial DNA and 15 microsatellites markers) and spatial analyses to evaluate how environmental (isolation by environment) and geographic factors (isolation by distance; and isolation by resistance) explain the observed patterns of gene flow between the study lineages.

Our results show clear patterns of introgression between both reproductive modes and lineages, which is largely reduced at higher elevations, limiting fire salamander dispersal at both sides of the Cantabrian Mountains.

Inferring the shallow phylogeny of true salamanders (*Salamandra*) by multiple phylogenomic approaches

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The rise of high-throughput sequencing techniques provides the unprecedented opportunity to analyse controversial phylogenetic relationships in great depth, but also introduce the risk of being misinterpreted by high node support values influenced by unevenly distributed missing data or unrealistic model assumptions. We compare the performance of three largely independent phylogenomic data sets to reconstruct shallow phylogenetic relationships in true salamanders of the genus *Salamandra*: (1) 3070 nuclear protein-coding genes from RNAseq; (2) 7440 loci obtained by RADseq; and (3) full mitochondrial genomes. In concatenation approaches, RNAseq and RADseq data retrieved fully congruent and supported

topologies. *S. infraimmaculata* was sister group to all other *Salamandra* species, *S. algira* sister to *S. salamandra*, and *S. atra* sister to *S. lanzai*. The phylogeny inferred from the mitochondrial genome sequences differed from these results by strongly supporting *S. atra* and *S. corsica* as sister taxa. Gene jackknife support for the *S. atra* - *S. lanzai* node in the concatenated analysis stabilized only with very large concatenated data sets. The phylogeny of true salamanders thus provides a compelling example of how classical node support metrics such as bootstrap and Bayesian posterior probability can provide high confidence values in a phylogenomic topology even if the phylogenetic signal for some nodes is spurious. Yet, the general congruence among the topologies recovered from the RNAseq and RADseq data sets increases our confidence in the results, and validates the use of phylotranscriptomic approaches for reconstructing shallow relationships among closely related taxa.

Importance of the Carpathian Basin in phylogeography of amphibians

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The Carpathian Basin is a complex transitional zoogeographic and climatic area located between the Carpathian Mountains, Alps, Dinarids and the Balkan Mountains. In the last decades it has been recognized as one of the important extra-Mediterranean refugial centres for several continental species. Phylogeographical analyses proved the importance of the region for amphibians as well. Concordant phylogeographical patterns were observed for mountain species with limited distribution in the area, such as *Salamandra salamandra*, *Ichthyosaura alpestris* and *Bombina variegata*. Different colonization routes from the Alps and the Carpathians were confirmed by the analyses of mitochondrial DNA fragments and several microsatellite loci, the Danube river serving as geographical barrier.

While some populations of *I. alpestris* might have survived the Last Glacial Maxima (LGM) in Bakony Mts. in northwest Pannonia, evidence for glacial survival of *S. salamandra* in the temperate forests of northeast Pannonia was found. Typical lowland species, such as *Triturus dobrogicus* and *Bombina bombina* may have used the Danube river as dispersal corridor to colonize the Pannonian lowlands. While *B. bombina* existed during the LGM in Mediterranean refugia, survival for *T. dobrogicus* was predicted for northwest and southwest Pannonia along the Danube and Sava rivers.

A review on distribution and genetic patterns of nearly all amphibian species occurring in the Carpathian Basin will be given using both published and unpublished data.

Ontogeny of head shape in *Triturus* newts: geometric morphometric approach

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We applied landmark based geometric morphometrics to capture head shape and to explore ontogenetic trajectories of head shape of two species from the genus *Triturus*, *T. ivanbureschi*, *T. macedonicus* and their F1 hybrids. *Triturus ivanbureschi* and *T. macedonicus* hybridise in nature and form a relatively wide hybrid zone.

Larvae of *T. ivanbureschi* (n = 25), *T. macedonicus* (n = 25) and *T. ivanbureschi* mothered F1 hybrids (n = 25) were obtained from experimental crossing and raised under controlled laboratory conditions. Their head shape was examined at three larval stages which were determined according to Glücksohn (1932) staging table based on the limb development (stages 42, 50 and 56). To capture and to quantify head shape at each ontogenetic stage, the configuration of 23 landmarks was used. We estimated average head shapes and calculated divergence in head shape between *T. ivanbureschi*, *T. macedonicus* and hybrid individuals.

Significant divergence in head shape between species and hybrids was found at each analysed stage. The analysis of shape changes across ontogenetic stages indicates that the divergence of ontogenetic trajectories occurs at later stages. Our results show that ontogeny of head shape during larval development of two closely related species and their hybrids follows the hourglass model. Further studies that will include a wider range of ontogenetic stages will show how bi-phasic life cycle and abrupt changes during metamorphosis reflect on phenotypic trajectories of head shape.

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Conspecific attraction in a landscape of fear: How different sources of information about predation risk affect sociability in lizard

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Predation is an important selective force in the evolution of prey behaviour. Anti-predator behaviours fundamentally enhance survival influencing fitness in many organisms. Among strategies, grouping behaviour can decrease the probability to be preyed upon through a risk-dilution effect, but at the cost of increased parasitism and competition. A fine-tuned assessment of predation risk is therefore needed to achieve the best benefit-cost ratio. To do so, individuals may use different sources of information: private (i.e. from personal experience during their lifetime), social (i.e. from conspecific cues) and transgenerational (i.e. from the prenatal environment through maternal effects).

We experimentally assessed the interaction between maternal, personal and social information about predation risk on the sociability in common lizard, *Zootoca vivipara*. The risk of predation was manipulated over two generations in a full-crossed design: gestating females from the 1st generation were maintained with or without predator scent. After hatching, each clutch was divided in halves and raised with or without predator cues to obtain all combinations of treatments between the 1st and 2nd generation. Sociability of the 2nd generation was tested in individual terraria where lizards could choose between areas with or without conspecific odor from lizards previously exposed or unexposed to predation.

The results showed that the multiple sources of information interactively shape lizard sociability: social information is more influential when private and maternal information are conflicting. We recommend that multiple sources of information should be examined when studying anti-predator defenses.

Osteological and geometric morphometric comparison of five species of *Rana* and *Pelophylax* (Anura: Ranidae)

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A description of skeletal development is scarce, in the generally well-studied family Ranidae. We provide an osteological description for the larvae of two species of *Rana* (*Rana tavasensis* and *R. macrocnemis*) and three species of *Pelophylax* (*Pelophylax ridibundus*, *P. bedriagae* and *P. caralitanus*).

Data are based on the cleared and double-stained specimens of 20 Gosner developmental stages (26-46) of each ranid species. Our results show that there are remarkable differences between the water (*Pelophylax sp.*) and mountain (*Rana sp.*) frog species. These are as follows: (1) the larval upper jaw, (2) the hyobranchial skeleton, (3) the ossification time and sequence, and (4) geometric morphometric differences.

A detailed description of larval osteological features of ranids conform with current phylogenetic position based on molecular and morphological data and obtain a remarkable information that can be important for interspecific relationships within the genus *Rana* and *Pelophylax*.

Secondary contact and limited hybridization of two distinct *Proteus anguinus* lineages in the underground of the Classical Karst

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The olm, or proteus, (*Proteus anguinus*; Urodela: Proteidae) inhabits subterranean waters of the Dinaric Karst. Recent phylogeographic studies based on mitochondrial DNA have shown that the taxon is subdivided into at least seven deeply diverged lineages (Gorički & Trontelj 2006, Trontelj et al. 2009). Apparently, all those lineages are geographically defined, allopatric and concordant with different karstic drainages. However, as we undertook more extensive sampling and analyses of variable nuclear DNA, we found some individuals that genetically belonged to the Ljubljana River lineage occurring also outside this drainage basin, reaching into the range of another lineage. Syntopic co-occurrence was found in the subterranean Reka (Timavo) River, in the northwester part of the proteus range.

We applied 21 newly developed microsatellites to genotype the individuals in the zone of mixed occurrence and search for signs of hybridization. All loci were amplified on samples from eight localities in the vicinity of the site with co-occurring lineages.

Bayesian clustering using the STRUCTURE software clearly distinguished between individuals from the Ljubljana drainage and the northwestern-most populations from Italian Carst, but detected F1-hybrids in the cave of Sežanska reka. The hybrid zone seems to be rather narrow, but its extent still needs to be investigated. Two plausible explanations for the crossing of subterranean hydrological boundaries by proteus are (1) occasional groundwater connectivity between different karstic basins during exceptional hydrological conditions, and (2) introduction of animals from the Postojna Cave to the Škocjanske jame for touristic purposes.

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New mitochondrial haplotype from *Vipera berus* group in Southern Romania

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The territory of Romania is a part of the Balkan biodiversity hotspot. Due to southern position Balkan peninsula served as refugium for many taxa in European biota in past climate fluctuations and hosts many endemic taxa, which split even before Pleistocene. The group of *V. berus* in the Balkans and adjacent Turkey is represented in addition to nominative subspecies *V. barani*, *V. b. bosniensis*, *V. b. nikolskii*. These four main groups have equal genetic distance to each other in spite of the different taxonomic level they are assigned. Currently, only two subspecies of the common adder are known in Romania – *V. b. berus* and *V. b. nikolskii*. The presence of *V. b. nikolskii* along southern slope of Carpathians was predicted in the paper of Zinenko et al. 2010.

While screening populations of *Vipera berus* in Romania, we found three individuals in a broad-leaved forest in Dâmbovița County, which appears to have a mitochondrial DNA haplotype, never found before (sequences of Cytochrome b and COI genes were analyzed). This new lineage has the same level of divergence in mitochondrial genes as the rest main lineages of the *Vipera berus* complex from each other, but share same alleles of two nuclear genes Bach and Rag 1. Distribution of alleles of these genes in Romanian populations is provided and the distribution of *V. b. nikolskii* in Eastern Romania is updated.

Demographic decline and population viability analysis of the Souss valley tortoise (*Testudo graeca soussensis*) in a degraded arid steppe-land of west-central Morocco: implications for conservation and management

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The Endangered Moorish tortoise, *Testudo graeca*, is the unique terrestrial chelonian species in Northwest Africa (Schleich et al., 1996). In an overgrazed arid steppe-land of west central Morocco, one of the main populations of the endemic subspecies, the Souss valley tortoise, *T. g. soussensis*, has been declining during the last decade due to a combination of climatic and human factors. The spring population size considerably decreased to more than 50% in less than 10 years with a mean density of less than 3 vs. 6 individuals/ha in spring of 2003 (Benkaddour et al., 2005). In spring of 2012, the population structure exhibited a male-biased sex-ratio (61:39) and a scarcity of juveniles (< 5%).

A Mark-Recapture programme from 2008 to 2012 allowed estimating population size, sex-ratio, and survivorship. Four major conservation problems in the study area were identified: i) captures for pet trade, ii) direct disturbance, iii) increased extinction risk due to small population size, and iv) habitat destruction. Using the VORTEX software, a population viability analysis was carried out on the basis of the last known population size, demographic and life history parameters from published data (including those obtained on the same population), indicated that without any management action, the population would go extinct during the forthcoming 20 years.

The most efficient management option for a long-term persistence of the population would be reducing the nest and neonate mortality. A management plan is proposed to minimize the impact of the threatening factors along with the reinforcement of the population and habitat restoration.

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Initial study on polymorphism of sex linked gene *CHD* in Serpentes suborder

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Several methods of gender identification in snakes are known. The simplest of them is based on the visible phenotypic differences like body sizes, relative length and width of tale, presence or sizes of anal spurs (Boidae) reflect sexual dimorphism. Other – practiced by experienced breeders include inversion of hemipenises by pressing abdominal part of tale or insertion of probe into cloaca.

The aim of our study was to check whether the *CHD* gene is suitable to detect sex of snakes as it is widely practiced in birds (Griffiths et al. 1996, Griffiths & Korn 1997). DNA of different species of snakes belonging to families Colubridae, Boidae and Viperidae was extracted from fragments of sloughing skin or tissue (from dead specimens). The PCR reaction with primers P2 and P8 (Griffiths et al. 1998) was done. After amplification, samples were subjected to direct sequencing by Big Dye Cycle Sequencing Kit (Applied Biosystems) and visualized in ABI 3500 analyzer (Applied Biosystems). Consensus sequences for each individual were established with usage of Mega 7 (Kumar et al. 2016). More than 105 polymorphic sites due to deletions, insertions and substitutions were detected. High level of inter taxonomic polymorphisms on the species level let us construct snakes' phylogenetic tree based on *CHD* gene. Contrary to birds, no sex linked polymorphism was found.

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POSTER PRESENTATIONS

Geographic morphological variability in northern Iberian vipers (*Vipera aspis* and *V. seoanei*) and its relation to environmental factors

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Environmental gradients along species distributions can lead to geographic variability in morphological traits. We investigated the geographic occurrence of distinct head ecomorphologies, and tested how they are affected by environmental parameters in two Iberian vipers: *Vipera seoanei* and *V. aspis zinnikeri*.

We measured head dimensions (head length, width and height, mouth length, and snout height) from 399 *V. seoanei* and 276 *V. aspis* individuals, where every character was first size-corrected through a lineal regression on SVL. In addition, we used geometric morphometrics (GM) to quantify head shape by recording the Cartesian coordinates of 29 landmarks in 153 *V. seoanei* and 94 *V. aspis* individuals. We investigated the spatial variation of head biometrics and shape through spatial interpolations, and tested for associations with environmental conditions.

Although we did not find any geographically coherent spatial pattern in head dimensions for head morphology in either species, head biometrics and shape were correlated to environmental conditions in both species. In *V. seoanei* significant correlations were found between head length and precipitation, and mouth length with temperature. However, no correlations were found between head shape and environmental factors. In *V. aspis*, snout height of females was correlated with almost all climatic variables. Head shape exhibited correlations with temperature and vegetation productivity. Our results suggest that head biometry and shape are distinctly affected by environmental factors in both species, likely representing alternative strategies to cope with environmental gradients.

Larval monitoring of the fire salamander (*Salamandra salamandra*) within the sand- and limestone areas of Salzburg with focus on aquatic and terrestrial habitat parameters

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The main distribution area of the fire salamander (*Salamandra salamandra*) in the state of Salzburg (Austria) comprises the flysch and northern limestone regions, basically favoring humid mixed beechwood forests with moderate to intense shading. Since adult fire salamanders are quite cryptic fellows, monitoring and assessment of the population size is difficult. Beside mark-capturing of adults, counting of larvae has been broadly applied to proof an increase or decrease of local populations. Concerning the higher detection ability of larvae this might be useful, but experiences in standardised monitoring of larvae are scarce.

The aims of our study were: (1) to compare larval abundances of sand- and limestone area (2) to estimate habitat parameters that effect larval abundances (3) to point out which aspects are important to develop an economic standardized method in terms of conservation.

From April to September 2016 two sites of each the pre-alpine flysch area and the limestone area were chosen to perform a day/night counting of larvae of 8 stream sections. Additionally measurements of abiotic parameters were included. Results show that 1) there were no significant differences in larval abundances due to geology, although RandomForestAnalyses showed a clear separation based on the structural elements of each stream surveyed in the field. 2) Number of potholes ($p=0.00906$) and proportion of calmed zones ($p=0.03973$) had a significant effect on larval abundance. 3) Overall counting of larvae at night definitely was more efficient. On average abundances of night counts were 6 times higher than day counts.

Determination of age, longevity and age at reproduction in two populations of the Beyşehir frog *Pelophylax caralitanus*

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Age determination provides crucial information on demographic parameters of amphibians' life-history. We studied age structure and growth in two populations of the Beyşehir frog, *Pelophylax caralitanus*, an endemic species of Turkey, distributed only in the Lakes District and its close vicinity in Anatolia.

Results showed that there is a statistically significant difference between body size and sexes (*Student t test*: $t=2.94$; $df=62$; $p=0.005$), and females are larger (the mean SVL= 82.77 ± 2.736 mm for female; 71.69 ± 1.693 mm for male) than males in both populations. Age structures differed significantly between populations in both sexes. However there is no statistically significant difference between age structure and sexes. The median age was 6.5 years and 4 years for males while for females, the median age was 7 years and 4 years for Burdur and Işıklı lakes populations respectively. Age at first reproduction was estimated as two years for Işıklı population and three for Burdur population in both sexes. Longevity of males was eight years in Işıklı population and nine years in Burdur population whereas in females it was 9 years in Işıklı and Burdur populations. Snout-vent length (SVL) and age were positively correlated both sexes and populations. These results show that there is intraspecific variation in age structure and body sizes between two Beyşehir frog populations.

Turkish Amphibians – Current status, threats and Conservation Implications

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Taxonomy and systematics are highly dynamic aspects of the biological sciences. The taxonomic research on Turkish amphibians has increased the last two decades with new techniques in science such as molecular, bioacoustics, and geometric morphometry techniques.

We reviewed the literature on Turkish amphibians with the recent changes in the taxonomic status. According to our results, there are 32 amphibian species living in and 11 endemic to Turkey. Two of these species are listed as Critically Endangered (CR) and 5 are listed as Endangered (EN) according to IUCN red list. There is a decrease in population trends for 21 amphibian species in Turkey.

In many cases, effective conservation of amphibian populations is limited by the lack of species-specific ecological knowledge, and by the lack of information on population structure. From our literature review, we determined general common factors across species that play roles in population decline in Turkey. Destruction of forest through commercial harvest and forest fires, construction of roads, dams, urbanizations, agricultural activities, pollution, drought and drainage can lead to either habitat destruction/loss or direct effect on species killing. In addition, over collection of specimens for scientific purposes, pet trade and food trade are the other major factors that are correlated with amphibian decline.

Conservation of the yellow-bellied toad in Gesäuse National Park: collecting baseline data

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In 2004, the yellow-bellied toad, *Bombina variegata*, was first recorded in Gesäuse National Park in the Northern Calcareous Alps in Styria (Austria). In summer 2016, we conducted a recapture study and collected biometrical data, to estimate population size, population structure and body condition.

Population size may be below 100 adult individuals, distributed over several disjunct sites, mostly situated at altitudes between 1000 and 1300 m. In one study area, adult survival was high (0.62) from 2010 to 2016. Body condition, assessed with the Scaled Mass Index, was positively correlated with altitude. High condition values may reflect both favourable growth conditions and limited opportunities to invest energy reserves in reproduction. Suitable breeding habitats are rare, originating from trampling of cattle, wallowing of deer or human activities. Syntopic Alpine newts, *Ichthyosaura alpestris*, and common frog tadpoles, *Rana temporaria*, may prey on spawn and larvae of *B. variegata*. Nevertheless, successful reproduction was observed at two sites in 2016. Tests for the presence of chytrid fungus, *Batrachochytrium dendrobatidis*, with skin swabs were all negative ($n = 13$).

Molecular detection of cryptic lineages of *Pelophylax* spp. in Sardinia

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Sardinian herpetofauna represents an extremely valuable resource in terms of biodiversity, due to its ecological and evolutionary uniqueness, resulting from long-lasting isolation from the mainland. Among amphibians, six species are endemic to the Island, the Sardinian mountain newt (*Euproctus platycephalus*) and five species of cave salamanders (*Speleomantes flavus*, *S. genei*, *S. imperialis*, *S. sarrabusensis* and *S. supramontis*), while other species occur also in Corsica and on few North-Tyrrhenian islands, the Tyrrhenian painted frog (*Discoglossus sardus*) and the Tyrrhenian tree frog (*Hyla sarda*). The Balearic green toad (*Bufo balearicus*) is thought to be autochthonous to the island, although it similarly occurs elsewhere (Poggesi et al. 1995). In contrast, water frogs (genus *Pelophylax*), supposedly introduced on the Island relatively recently, were found in some sites of north and south Sardinia, and were assigned to *Pelophylax* cf. *ridibundus* on morphological basis.

As part of an extensive project aiming to assess the status of Italian water frog populations (Bellati et al. 2012, Domeneghetti et al. 2013), we isolated diagnostic mitochondrial markers (ND3) in order to: characterize allochthonous populations; infer their putative geographic origin; search for the co-occurrence of distinct cryptic lineages (*P. ridibundus*, *P. kurtmuelleri*, and *P. bedriagae*) at the same site attesting multiple introductions.

According to results, both *P. kurtmuelleri* and *P. bedriagae* haplotypes were detected. The latter could be considered naturalized in Sardinia, as eggs have been assigned to this lineage. Although the persistence of *Pelophylax* may be due to the occurrence of vacant niches in Sardinia, the dry hot season may not favor its expansion along rivers.

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Interpopulation and Seasonal variation in the feeding habits of the Moroccan Spiny-tailed lizard *Uromastix nigriventris* Rothschild & Hartert, 1912 (Sauria: Agamidae)

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Food habits of the Moroccan spiny-tailed lizard, *Uromastix nigriventris*, from three distant localities along a latitudinal Mediterranean-Pre-Saharan gradient from north-east to southwest of the Atlas range, Morocco, were investigated in spring and autumn 2015 using fecal microhistological analysis (Baumgartner & Martin, 1939; Dusi, 1949).

The obtained results show, that these lizards are predominantly herbivorous feeding on just 4 to 13 different plant species depending on locality and season. However, they also eat some insects, namely coleopterans and ants (Formicidae) (up to 6%). They heavily ingested annual and perennial herbaceous plants through a single season. There were significant differences among seasons in terms of Margalef's richness and Shannon diversity indexes. The highest values of species richness and diversity were observed in the Pre-Saharan locality (Fask) especially in spring. Dietary overlap was low between Saka (the Mediterranean locality) and Skoura (Dades valley south of central High Atlas range) in spring. Conversely, there was substantial overlap in the diets in autumn with a more pronounced similarity between Saka and Skoura.

A synthesis of published information on food habits suggests that most of the studied extant *Uromastix* species, depend also on a few species of plants although differing in composition of core diets.

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Notes on the breeding activity of *Rana dalmatina* in a rural area.

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Female breeding activity and tadpole development of *Rana dalmatina* were monitored in a rural area in Central Italy. The distribution of the breeding sites and data on the phenology of this species were recorded in order to suggest possible conservation measures. Clutches were searched for in watercourses characterized by different depth, width, vegetation features, and located at different distances from woodlots.

Spawning took place in ditches without vegetation and shade, as well as in aquatic habitats rich in semi-aquatic plants and with trees and bushes on their banks. The spawning activity occurred from mid-February to mid-March, with a peak in the first two weeks. The first tadpoles were observed after three weeks from deposition. The mean number of eggs per clutch was few less than 600 and the number varied among watercourses. Apparently, all clutches developed, despite the presence of the crayfish *Procambarus clarkii* in the area. However, most of the watercourses dried up before tadpoles' metamorphosis was completed, thus causing the loss of about 46% of the clutches. The only site that guaranteed the complete tadpoles' metamorphosis, and in which the highest density of egg-batches was recorded, is the watercourse located next to a woodlot, characterized by semi-aquatic plants and semi-natural vegetation on its banks.

Our results confirm that the conservation of natural and semi-natural habitats, as vegetation buffer zones along watercourses and woodlots, are key elements for amphibian conservation in agricultural landscapes.

Moving Away from the "Cave Lab Paradigm": Successful Captive Breeding and Raising of the European Blind Cave Salamander *Proteus anguinus* using "Optimal Artificial" Conditions

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The European blind cave salamander (*Proteus anguinus*) has long been of special importance as a symbol of natural heritage and conservation as well as for its potential as a model organism in evolutionary developmental studies of cave adaptations. Unfortunately, attempts to breed this salamander in captivity have had only limited success. Most such attempts have followed the "cave lab paradigm", utilizing caves that have been converted to labs or vice versa. The only cave lab that successfully established a sustainable breeding program of *Proteus* for any length of time is the cave laboratory of the CNRS established in 1948 in Moulise, France. The Tular cave in Slovenia, modeled after the Moulis cave lab, has also had some limited success in breeding and raising *Proteus* in captivity. But none of these cave labs have been successful over the long term, with slow and unpredictable breeding events and low (< 50%) survival rates to hatching (Juberthie et al. 1996).

A recent accidental egg-laying event by a single *Proteus* female at the Postojna Cave provided the opportunity to experiment with "optimal artificial" (OA) conditions achieved by optimizing three fundamental requirements for captive salamanders: 1) clean water, 2) correct temperature, and 3) high quality (live) food. Approximately 92% of the *Proteus* embryos raised under OA conditions have survived so far. The Postojna case represents an important step in moving away from the "cave lab paradigm", which has dominated the field for decades and which has had only limited success, to implementing OA conditions for raising *Proteus* in captivity.

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Opportunistic pathogens of the captive specimen of the European blind cave salamander *Proteus anguinus*

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As other amphibians kept in artificial or semi-natural environments, the specimens of troglomorphic *Proteus anguinus* are susceptible to opportunistic microbial infections, due to the suppressed defensive mechanisms of the host exposed to stress or suboptimal living conditions. Despite absence of published data on infection of *Proteus* in captivity, several infections were observed in our lab over the years including entamoebae, water molds from the genus *Saprolegnia* sp. and bacterium *Aeromonas hydrophila*.

Herewith we present a case report of an infection of *Proteus* specimen kept in captivity for 6 years, with an opportunistic pathogenic black yeast. The gross clinical signs of *Proteus*'s specimen comprised an excessive mucus production, sloughing of epithelial cells and skin ulcers. The histopathological analysis based on light and electron microscopy showed scattered pigmented hyphae and yeast-like cells in almost all sampled tissues. Beside the skin, the kidney were the most affected organ, showing tissue disorganization, extensive necrosis and large aggregations of inflammatory cells. The differential count of leukocytes on blood smears revealed the prevalence of monocytes (46 %), followed by the lymphocytes (28,6 %), neutrophils (24,4%) and eosinophils (0,8%), while basophils were not observed. Sampling of the ulcer resulted in the isolation of bacterial species belonging to the genera *Acinetobacter*, *Pseudomonas* and *Shewanella* and fungal species *Exophiala salmonis*, *Candida atlantica*, *Fusicolla aquaeductuum* and *Acremonium alternatum*. Polymorphic black yeast *E. salmonis*, known as an opportunistic fish pathogen, was recognized as the primary infection agent. This is the first report on a fungal infection of *Proteus* and on cave salamanders in general.

The Genetic Investigation of *Ablepharus* Lichtenstein, 1823 (Sauria: Scincidae) Genus in Turkey

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The genetic composition of Anatolian populations of the genus *Ablepharus* were studied by using two mitochondrial (COI and cytB) and one nuclear (c-MOS) gene regions. All the analyses were carried out with 40 specimens from eight taxa of genus *Ablepharus*.

After total genomic DNA extraction, totally 2185 bp of gene was amplified for each specimen and the amplification products were sequenced in both directions with the primers used in the PCR reactions. The generated dataset was used in both network and phylogenetic analyses. Haplotype and nucleotide diversity were 0.999 and 0.124 for cytB, 1.000 and 0.130 for COI, and 0.830 and 0.008 for c-MOS, respectively. The Bayesian Interference methods supported five main clades: *A. budaki*, *A. bivittatus*, *A. chernovi*, *A. chernovi eiselti* and *A. kitaibelii* with high support values. All haplotypes of *A. bivittatus* and *A. chernovi* settled in one subclade, while the haplotype of *A. budaki*, *A. chernovi eiselti* and *A. kitaibelii* grouped in two subclades. The Bayesian tree was also supported by network analysis.

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Cryoresistance of three toad species from North Asia

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Geographic ranges of many Anura in North Asia include cold regions with deeply freezing soils. It is thought that, compared to frogs (Ranidae) and tree frogs (Hylidae), toads (Bufonidae) are not cold resistance (Swanson et al., 1996; Voituron et al., 2009). To clarify the parameters of cryoresistance of three toad species (*Bufo bufo*, *B. raddei*, *B. viridis*), we determined the supercooling point (SCP) as well as the thresholds of tolerated temperatures.

Mean SCP values were: for *B. viridis*, $-3.3 \pm 0.2^\circ\text{C}$, for *B. bufo*, $-2.9 \pm 0.3^\circ\text{C}$, and for *B. raddei*, $-2.5 \pm 0.1^\circ\text{C}$. The temperature of -1°C was tolerated by all three studied species in supercooled condition. The exposure of 7 days was sustained by all individuals of *B. viridis*, 30 days - all individuals *B. bufo* and *B. raddei* (*B. viridis* were not tested for more than 7 days). At -1°C , ice crystals were formed on the substrate as well as on toads' body surface, without causing inoculation as described for *Bufo cognatus* and *B. woodhousei* (Swanson et al., 1996). The temperature of -3°C for the period of 3 days was not tolerated by any investigating individual toads.

It is therefore reasonable to assume that toads in North Asia spend winter either inside water bodies, like the grey toad in Scandinavia (Laitinen, Pasanen, 1998) or *B. gargarizans* on the Sakhalin Island (Flyaks, 1991); or in the areas warmed by various subterranean water sources, like *B. boreas* (Campbell, 1970); or burrow into the ground below freezing depth, as described for many species.

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The effect of thermal acclimation on hematological variables in the grass snake (*Natrix natrix*)

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A positive relationship between ambient temperature and the rate of physiological processes is well established for ectotherms. Higher aerobic metabolism under warmer ambient temperatures suggests also higher demand for oxygen supply through the blood. We investigated if acclimation to different ambient temperature affects hematological variables (HVs: hematocrit, hemoglobin content, erythrocyte size and number) in reptiles. Grass snakes (*Natrix natrix*) were exposed for six months to either 18°C or 32°C before we blood sampled 14 individuals to determine HVs and the intensity of erythropoiesis by counting the frequency of immature erythrocytes.

All HVs showed significantly lower values in warm-acclimated snakes compared to cold-acclimated ones. Immature erythrocytes were significantly more abundant in warm-acclimated snakes. Our results confirm previous findings on reptiles showing negative effect of temperature on hematocrit and hemoglobin concentration and expand this pattern on erythrocyte count and size. Despite higher metabolic rate under warm temperatures lower hematocrit, hemoglobin content, erythrocyte number may be compensated by smaller erythrocyte size. Hematocrit and cell number positively affect blood viscosity, while erythrocyte size is negatively related to blood viscosity. Sufficient oxygen supply may thus be secured by a trade-off between surface-area-to-volume ratio of the erythrocyte and the total cell fraction of the blood. Modulation of this trade-off may occur through modulation of heart rate known to be higher in high temperatures. Elevated erythropoiesis in warm-acclimated snakes together with lower cell number suggest increased erythrocyte turnover in high temperature. In summary, this response to acclimation to different ambient temperatures suggests fundamental adjustment of the blood.

The role of protected areas in health status of palmate newts

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Biodiversity conservation has traditionally been focused on protected natural areas. There are few initiatives to integrate environmental conservation management in the whole territory or to pay attention to areas outside the protected natural ones, and even less, to anthropized areas. However, much of the biodiversity is found in these last ones, where some species are even more abundant. Despite the global decline of amphibians and their vulnerability to pollution, some species, such as the palmate newt (*Lissotriton helveticus*), have successfully colonized anthropized areas (farm lands, forest plantations, urban areas). Their ability to use multiple types of aquatic habitats to breed and its presence in both terrestrial and aquatic habitats makes it an appropriate species to study whether habitat protection favors a better state of conservation of amphibians populations.

We captured male adult newts in ponds located in four Natural Parks and in corresponding four nearby unprotected areas (three ponds in each Natural Park or nearby area, and ten males in each pond). We measured their body condition and immune response by phytohemagglutinin test to characterize their “conservation health”. Both aspects are directly related to survival and reflect possible effects of habitat alteration, while immune response one is also related to increased vulnerability to emerging diseases. We have found that the degree of anthropic habitat, but not the degree of protection of the territory affect the immune response and, hence, to amphibian vulnerability.

Phylogenetic assessment of the Valentin's Lizard, *Darevskia valentini*, (BOETTGER, 1892) in Turkey

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We searched the phylogenetic relationship of populations for *Darevskia valentini*, distributed in Turkey, based on both mitochondrial (12S rRNA and cyt b) and nuclear markers (MC1R).

A total of 27 *D. valentini* specimens were used in this study. All DNA sequences were aligned separately for each gene using MAFFT v7. The number of haplotypes, haplotype diversity, and nucleotide diversity were calculated in DnaSP v5. The most suitable model of DNA substitution was chosen using jModelTest v2. Neighbor-joining and Maximum Likelihood were performed with MEGA v7 and RAxML v7, respectively. Bayesian Inference was conducted in MrBAYES v3 using the suitable models of evolution for each gene regions. The genealogical relationships among the 12S, cyt *b* and MC1R haplotypes, separately, were constructed using TCS v.1.21.

In total, 355 bp of 12S, 389 bp of cyt *b* and 608 bp of MC1R were obtained. For 12S and cyt *b*, 7 and 13 haplotypes were detected and parsimony informative sites were 8 and 44, respectively. Phylogenetic analyses (NJ, ML and BI) produced trees with similar topologies including two well supported clades. The first consists of populations came from Ağrı, Erzurum and Van regions and the second is related to Kars, Ardahan, Bayburt, Gümüşhane, Sivas and Kayseri. Our results showed surprisingly that almost all clades are of high statistical values (bootstrap or pp) on the external nodes. In conclusion, this study disclosed new genetically lineages of *D. valentini* constructing its elaborated phylogeny for the first time.

The Relation between Body Size and Egg Volume of Population of *Heremites vittatus* (Olivier, 1804) (Squamata: Scincidae) in Different Elevation in Turkey

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Few studies have investigated how lizards at different elevations exhibit a relationship between female body size and egg volume. In this study, we researched this concept on the populations of *Heremites vittatus*, the Striped Skink.

We measured the volume of 455 eggs of *H. vittatus* that were obtained from three different elevations (0-500m, 500-1000m, 1000m above) in Turkey. The volumes, SVLs and elevation values were analyzed at SPSS v24.

As a result, female eggs at high-elevation are smaller in volume than those of low-elevation females. However, egg volume does not have any correlation with female SVL. Female SVL is not significant among three different elevations. Such studies on lizard have provided to understand the life history of lizard among different elevations.

Molecular detection of cryptic alien hybridization in wild populations of waterfrogs (*Pelophylax* spp.) in northern Italy, and their effects on population structure and reproduction

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Frogs of the genus *Pelophylax* are widespread in Eurasia and can be identified only on a molecular basis due to the absence of clear diagnostic features. These species have been much studied for their reproductive mechanism in which sympatric hybridization between genetically distinct parental species produces diverse genetic forms of viable hybrids (hybridogenesis, Holsbeek and Jooris, 2010). The most common hybrid is *P. esculentus* carrying the genomes of *P. ridibundus* and *P. lessonae* (Uzzell and Berger, 1975), which commonly excludes in his germinal cells the *P. lessonae*-DNA and transmits the other one clonally, only persists in the absence of *P. ridibundus*. Recently, the equilibrium of this system was disrupted by the anthropogenic introduction in Western Europe of alien *P. ridibundus* (Schmeller et al., 2007).

Our aim is to adopt a two-step approach involving (1) the analysis of genetic structure of “polluted” vs. “pure” populations; (2) the production of *in vitro* crossing experiments between autochthonous vs. allochthonous lineages, to assess the fitness contribution of each parental genome in hybrid offspring.

To this end, we screened 10 microsatellite loci in several populations in Northern Italy to infer population structure and isolate adult males and females of *P. esculentus*, *P. lessonae*, *P. kurtmuelleri* and *P. ridibundus*. Pure lineages were crossed adopting Berger’s protocol (1994) with minor modifications. Results show both the occurrence of “polluted” hybrids in the wild and alternative outcomes of hybridizations in captivity, in terms of tadpoles’ development and survival, as a proxy to quantify the potential of “polluted” hybrids to local adaptation (Ebert et al., 2002, Fitzpatrick and Schaffer, 2007).

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Insight into the thermal ecology of the meadow lizard (*Darevskia praticola*) in Serbia

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Thermoregulation affects all aspects of reptilian biology, such as physiology, reproduction, and ecological performance. Investigating thermoregulation in less favourable (e.g. peripheral) habitats could be an important tool in predicting the impact of global climate change and the risks of local extinctions.

We studied the thermal biology of the meadow lizard (*Darevskia praticola* EVERSMANN 1834) in the peripheral part of the distribution range (western edge of the distribution area) and its local temperature requirements. In the study we applied the experimental protocol designed by Hertz et al. (1993), to determine whether lizards actively thermoregulate, to estimate accuracy and effectiveness of thermoregulation, and to evaluate the thermal quality of the habitat.

Selected body temperature range (T_{sel}) of the meadow lizard under controlled laboratory conditions was 27.1–31.1°C, with an average of $29.2 \pm 0.2^\circ\text{C}$. In the field, active body temperatures (T_b) averaged $29.0 \pm 0.4^\circ\text{C}$, and operative temperatures (T_e) (environmental temperatures available to lizards) averaged $25.6 \pm 0.2^\circ\text{C}$. Large proportion of T_e s (63.6%) fell below the T_{sel} range of the meadow lizard, while the 62.3% of T_b readings fell within the T_{sel} . Lizard T_b s were substantially closer to the species' T_{sel} range, with mean deviations of T_b s from T_{sel} ($\bar{d}_b = 0.7$), than were T_e s, with mean deviations of T_e s from T_{sel} ($\bar{d}_e = 3.3$). Obtained values of thermoregulatory indices suggest that the meadow lizard actively thermoregulates, with high accuracy (average difference between T_b and T_{sel}) and effectiveness (E) (the extent to which T_b s are closer to T_{sel} than T_e s are to T_{sel}).

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Primary myogenesis in amphibians and reptiles – comparative studies

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Myogenesis is a multistep process in which mononucleated myoblasts fuse to form multinucleated myotubes. Following the fusion multinucleated myotubes differentiate into muscle fibres. Many different lines of evidence indicated that in all vertebrates the mature muscle fibres are multinucleated. Comparative studies on myogenesis in vertebrates revealed that early developmental pattern of multinucleate muscle formation varies between vertebrate taxa.

In our studies we compared amphibian myogenesis: *Xenopus laevis* (Anura) and *Triturus vulgaris* (Urodela) to reptilian myogenesis *Lacerta agilis* (lizard) and *Natrix natrix* (snake). Our studies revealed that during early steps of *X. laevis* myogenesis mononucleated myoblasts without the fusion differentiate into morphologically and functionally mononucleated myotubes whereas in *T. vulgaris* multinucleate myotubes are formed directly in trunk part of the embryo. Surprisingly, we observed mononucleated myotubes in the caudal part of *T. vulgaris* embryo. In reptiles, both *L. agilis* and *N. natrix*, an intermediate stage of myogenesis, leading to multinuclearity of muscle fibres, is the formation of mononucleated myotubes. The growth of muscles in all studied species was due to the participation of mesenchymal cells which migrated into the myotomes between mononucleated or multinucleated myotubes. By the use of Pax3 and Pax7 (markers of muscle progenitor cells) immunodetection we confirmed that in reptiles the dermomyotome is a source of muscle precursors. The authors believe that the presence of mononucleated myoblasts which differentiate into mononucleated myotube during primary myogenesis in amphibians and reptiles could be a plesiomorphic feature of vertebrate myogenesis.

Do blood parasites mediate the interaction between the lacertids *Iberolacerta horvathi* and *Podarcis muralis*?

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Parasites are important drivers for shaping populations; they can significantly affect clutch size, hatching success, and individual's survival, among others. In ecologically similar host species shared parasites may shape the interspecific interaction.

Our goal is to investigate the role of parasites in shaping populations of two lizard species: *Iberolacerta horvathi* and *Podarcis muralis*. We predict that if parasites affect both species differently, the intensity of parasitism will be lower in the dominant species under syntopic conditions but similar under allotopic conditions. If common parasites affect both species similarly the intensity of parasitism will be increased in syntopic vs. allotopic populations due to interspecific competition.

Lizards were sampled in four syntopic and three allopatric populations of both species. We characterized the prevalence and intensity of the parasite community using microscopy and genetic markers. Results were analyzed to test for the effect of species, geographic region and sympatry/allopatry. We will present the preliminary results collected in spring and summer 2017. This will be the first study regarding the potential impact of parasites on the interspecific interaction between these two species, and will contribute to a better understanding of the role of parasitism on host communities.

Tick infestation of three lacertid lizard species from different localities in Serbia -preliminary results

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To investigate host-parasite relations in different habitats and its influence on host fitness a total of 132 lizards belonging to three lacertid species (*Lacerta agilis*, *Podarcis muralis*, *Podarcis tauricus*) were collected at three localities in Serbia from March to June of 2017. First locality (Hatarice, 90 m a.s.l.) is a rather preserved steppe habitat of *P.tauricus*, 66 km north-east of Belgrade. Second is a montane meadow at Divčibare (980 m a.s.l.), a habitat of *L. agilis*, 115 km south-west from the capital. Both of them have low anthropogenic disturbance. Third location (Reva, 70m a.s.l.) is a suburban area, just outside Belgrade, inhabited by *L. agilis* and *P. muralis*, with a high level of anthropogenic disturbance.

Lizards were collected by noose or hand and examined for external parasites. After their body mass and SVL were measured, they were marked and released. A total number of 117 ticks were removed and preserved. The percentage of tick infestation in every population was determined. Lizard's body condition index, parasite load and percentage of infestation were calculated.

Lacerta agilis from Reva had the highest tick infestation overall (57.14%) and *P. muralis* from Reva were also highly infested (42.31%). Tick infestation was low in habitats with minor anthropogenic disturbance (Hatarice 2.38%; Divčibare 2.94%). Data analysis showed that body condition index was not significantly correlated with parasite load. No significant differences in infestation percentage were detected between two species found in the same habitat (Reva). However, habitat type had a statistically significant influence on tick infestation intensity among populations.

Tail regeneration in a lacertid lizard (*Podarcis erhardii*): effects of diet composition

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Tail autotomy is a defensive mechanism used by many lizards as a response to attempted predation or intra-specific aggression (Arnold 1984, Cooper et al. 2015). The tail is a vital organ associated with survival and reproductive success (Fox & Rostker 1982, Fox et al. 1990, Salvador et al. 1995, Fox & McCoy 2000) and thus caudal tissue regeneration shortly follows autotomy. The speed at which regeneration is completed varies across species and populations (Vitt et al. 1977).

The ecological factors that influence tail restoration in lizards have not yet been properly investigated. There has been evidence indicating that caudal regeneration is prioritised over other energy consuming procedures, even when resources are limiting (Lynn et al. 2013). However, the impact of diet quality on regeneration has not yet been assessed.

Here we examined whether tail regeneration rate is affected by diet composition in an insectivorous lacertid (*Podarcis erhardii*). Adult male lizards were randomly assigned to three different diet treatments in the lab: two distinct invertebrate mono-diets (*Tenebrio molitor* larvae, *Acheta domesticus* nymphs) and a highly diversified one (*Tenebrio molitor* and *Galleria mellonella* larvae, *Acheta domesticus* and *Blaptica dubia* nymphs). Autotomy was induced after a week and the regeneration of the tail was measured weekly until full restoration was achieved.

According to our results, the two mono-diets had no impact on tail regeneration: tails grew at the same rate, regardless of food composition. Nonetheless, the individuals that were fed with the multi-source diet deviated from the other two groups and regenerated caudal tissues at a faster pace.

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Detection of the *Chytrid*-fungus (*Batrachochytrium dendrobatidis*) in Vienna: Biomonitoring using environmental DNA

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There are many reasons for the worldwide amphibian decline since the 1950s, such as loss of natural habitats, climate change or environmental pollution. One of the most devastating causes is the pathogenic, aquatic zoosporic fungus *Batrachochytrium dendrobatidis* (*Bd*), which is responsible for the global disease chytridiomycosis in amphibians. In Austria infected specimens were detected in all nine provinces. For example in Vienna the fungus was detected in the year 2011 in 5 of 7 sampled sites. There is still no mass mortality provoked by *Bd* in Vienna but the quantity of *Bd* positive tested animals is alarming. Further observation of the fungus distribution is therefore important.

This study will review the practicability of the Environmental DNA method for a „*Bd*-monitoring“ in Vienna. DNA was extracted from water samples of 20 different water bodies in Vienna, which were collected over a period of two months. The samples are analyzed by Real Time PCR Methods. Additionally skin swabs were taken from amphibians around or in the water bodies. *Bd* positive tested specimens from the Vienna Zoo were included as positive control.

The influence of embryonic environments on development of *Bufo bufo* tadpoles

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In a series of experiments it was shown that there is a correlation between the morphometric characteristics of tadpoles and the conditions of their embryonic development. The growth parameters, such as ventral body length and dorsal body length, of *Bufo* tadpoles depend on the conditions of embryonic development (Dmitrieva, 2005, 2012). Tadpoles that came from eggs that developed in low density were significantly larger than those in the high density group. Tadpoles hatched from the eggs that developed in a stretched egg cord had much greater dorsal body length than those hatched from the loose egg cord.

In another series of experiments, eggs of the common toad had been placed in different oxygen concentration: oxygen deficiency and normal oxygen supply. After hatching tadpoles were split into groups of 2 and 5 individuals per 0,3L. The experiments were finished after the formation of metatarsal tubercle (st.36, Gosner, 1960).

The oxygen deficiency during embryogenesis influenced primarily formation of tadpole head characteristics: distance between the margins of the nostrils (Sp.n.), ratio Sp.n. to distance between centers of eyes (Sp.c.r), and ratio Sp.n. to body width (Wd.b). Tadpoles, which developed in conditions of oxygen deficiency, were significantly larger than those developed in normal oxygen supply in Sp.n./Wd.b and Sp.n./Sp.c.r. The influence of oxygen deficiency on the formation of Sp.n. varied between clutches: under high density of tadpoles in two clutches Sp.n. was larger in individuals that developed under conditions of normal oxygen concentration, and in the other clutches – in the oxygen deficiency conditions.

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Niche diversity in Neotropical poison frogs, genus *Ameerega*

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Poison frogs in the genus *Ameerega* are a group of 33 known cis- and trans-Andean species. We here concentrate on those from East of the Andes, which are suggested to constitute a monophyletic assemblage. Most of these species are found in lowland and montane rainforests along the lower eastern Andean versant and in the upper Amazon basin. Few taxa have dispersed into the lower basin and the Guyana shield where they occupy *terra firme* lowland rainforests. Another lineage has emerged into semi-humid forest and *cerrado* habitats of Bolivia and the Brazilian highlands. Comparatively few species occur in sympatry with congenetics.

These circumstances led us to assess inter-specific divergence of abiotic and biotic niche dimensions, represented by climate, habitat and body size characteristics. In this way, we identified (1) groups of allopatric species with relatively narrow niches that show both (i) niche overlap and (ii) niche divergence, and (2) species that occur in sympatry with others that display relatively large niches.

Concentrations of Heavy Metals in Tissues From Habitats of The Nile Soft-Shelled Turtle (*Trionyx triunguis*) in Turkey

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The concentrations of heavy metals (Al, B, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sn and Zn) were measured in carapace, nail, muscle and liver tissues of the Nile Soft-Shelled Turtle *Trionyx triunguis* (FORSKAL, 1775), collected from Kukurtlu Lake-Mugla, Paradeniz-Mersin, Mersin, Kazanlı- Mersin in Turkey.

The levels of heavy metals varied significantly among the tissues. Cd and Co concentrations were below the detection limit in tissue samples and Ni concentration was not detected except carapace, muscle and liver tissue from Kazanlı-Mersin sample. Cu concentration was detected only from the Kazanlı-Mersin sample and found a statically significant difference between the tissues. Fe and Al concentrations were detected high in all tissue samples. The mean concentrations in tissues were analyzed as Fe>Al>Zn>Cr>B>Sn>Ni>Mn>Cu>Pb.

No exact information about the metal concentration in tissues of *Trionyx triunguis* was available until this study. Generally, recorded metal concentrations were within the range or below the levels in turtles from global studies.

No macroscopic observable effects of environmentally relevant concentrations of a commonly used glyphosate-based herbicide on anuran metamorphosis

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Glyphosate-based herbicides are dominating the world market. There is an on-going debate on their environmental safety. Compared to other pesticide formulations, several amphibian toxicological studies have been conducted aiming at different potential effects of glyphosate-based herbicides. The results and as consequence the conclusions are varying and effects were beside dose-dependency specific for the species, the population, the life-stages and the formulations.

Because different studies have shown effects of glyphosate-based herbicides on anuran metamorphosis – e.g. due to endocrine effects on the thyroid axis – we here studied the impact of a commonly used glyphosate-based herbicide (Roundup® UltraMax) on metamorphosis of the Mediterranean Painted Frog (*Discoglossus pictus*). Effects of short and pulsed exposure of early premetamorphic and prometamorphice larvae to the herbicide formulation representing an environmentally relevant temporal application scheme were studied. In addition, larvae of all considered stages were repeatedly exposed. We applicated 0.9 and 2.25 mg active ingredient/L as test concentrations covering the worst-case expected environmental concentrations of glyphosate for surface waters in Germany and the European Union.

There were no toxic effects on macroscopic endpoints. The compound did not increase mortality or deformation rates or caused clinical signs or effects on time to metamorphosis. However, to validate the apparent environmental safety data on anuran metamorphosis, more information on and experiments with realistic contamination levels are necessary (e.g., peak concentrations, concentrations of added substances and not only of the active ingredient). Sufficient buffer strips between the farmland and amphibian ponds must be considered on the basis of future experiments and in connection with the susceptibility of the adults.

European Pond Turtles in Serbia – preliminary data on sexual size dimorphism and demography

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European Pond Turtles (*Emys orbicularis*) and their habitats have been receiving great attention of conservationists across Europe. In Serbia, this species is considered strictly protected, although its national Red List status was assessed as Data Deficient (Krizmanić & Džukić 2015). Population studies were initiated only recently, with the financial support of Rufford Small Grant Foundation, on the few localities across Serbia.

Here we present preliminary results on morphological differences and basic demographic characteristics of two studied populations at Ludaš lake on North of the country, where Capture-Mark-Recapture studies last since 2011, and Malo Crniće pond in Eastern Serbia explored since 2015. Studied habitats differ in size of the water body, composition of the surrounding vegetation and presence of anthropogenic pressures.

Recapture rate was approximately 38% on both populations. In general, European Pond Turtles have females biased sexual size dimorphism (Kaviani & Rahimibashar 2015, but also see Zuffi et al. 2006). At Ludaš population females are heavier and larger sex, according to all measured traits (shell length, width and height). On the other hand, in population from Malo Crniće sexual dimorphism is significant only in weight and shell height. Our sample shows that turtles from Malo Crniće, both males and females, grow to be larger and heavier comparing to those from Ludaš lake. This difference could be due to large eutrophication of the small isolated pond, along with additional nutrients such as carcasses from illegal dumping site next to the pond.

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First data on *Batrachochytrium dendrobatidis* infecting amphibian populations in National protected areas in Italy

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There are relatively few data on the prevalence of the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) in amphibian populations from Italy, where it has been detected in different species, comprising Sardinia (Bielby et al. 2013; Tessa et al. 2013). To assess the diffusion of *Bd* infection in amphibian populations of three Italian National parks, a molecular screening, part of the Italian Ministry of Environment project "Monitoraggio delle specie di ambiente umido acquatico", began in 2015.

Overall, 496 swabs from eleven species were analysed by RT-PCR, using a protocol based on Sybr Green chemistry and validated against the standard Taqman PCR assay of Boyle et al. (2004).

The preliminary results show that *Bd* infection, detected in 6 species, is present in all the three sampled areas with different prevalence. In particular, in the Cinque Terre National Park (NW Italy) the overall infestation rate was 3% (7 infested amphibians out of 227) and in the Circeo National Park (central Italy) 5% (10 infested out of 222). A higher infestation rate (32%, 15 infested out of 47) was observed in the Pollino National Park (S Italy). However, there were no evidences of unusual mortalities in any study area and the individual prevalence was low, ranging from 1 to 30 *Bd* genome equivalents per individual swab. This project aims to include more national protected areas, while enlarging the sample size of unstudied and under-represented species.

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A non-destructive approach for monitoring the health status in the European Blind Cave Salamander, *Proteus anguinus*: Differential counts of white blood cells from blood smears

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Because of its special biology and subterranean habitat in porous limestone, *Proteus* is thought to be extremely vulnerable to environmental pollution in addition to pathogens which are of global concern to amphibians, and so is considered “threatened” by the IUCN. For these reasons, developing non-destructive methods for monitoring the health status of populations is an important approach in the conservation biology of this unique species.

We used blood smears from *Proteus* specimens ($n = 3^*$) to study the detailed morphology of blood cells and for differential counts of white blood cells (WBC). This approach is useful in assessing the health state of amphibians (Davis et al. 2008, Barriga-Vallejo et al. 2015).

Our results show that *Proteus* is characterized by a wide range of variation in the morphology of the different types of blood cells. Mean blood cell counts showed 7.0 ± 5.4 % leucocytes (expressed as mean \pm SD), with 76 ± 19.7 % of the WBC being lymphocytes, 19 ± 16 % neutrophils, 2.7 ± 3.8 % eosinophils and 2.3 ± 0.6 % being monocytes. Basophils were nearly absent. The ratio of neutrophils to lymphocytes (N/L ratio) appeared low (0.20) indicating that the animals were not stressed during the blood sampling. Even though this research was done on a small sample size, we have established a very important basis for further hematological analyses. In particular, further studies are needed to obtain baseline values of WBC in *Proteus* in order to detect WBC/immunological response to possible environmental stressors.

*Obtained from Planina cave in SW Slovenia with the permission of the Ministry of the Environment and Spatial Planning, Republic of Slovenia, document No. 35601-1/2010-6.

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Geometric morphometric analysis of cranial skeleton in *Bufo bufo* (LINNAEUS, 1758) and *Bufo verrucosissimus* (PALLAS, 1814)

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The use of geometric morphometrics for studying phenotypic variation in amphibians and reptiles has increased in the last decade (Kaliontzopoulou 2011). In this study, we tried to explore the variation in skull size and skull shape of Common toad (*Bufo bufo*) and Caucasian toad (*Bufo verrucosissimus*) by using a geometric morphometric approach.

We analyzed 10 specimens of *B. bufo* from Bolu in the Western Black Sea Region and 10 specimens of *B. verrucosissimus* from Artvin in the Eastern Black Sea Region in Turkey. A set of 17 landmarks was digitized on both the ventral and dorsal sides of each skull using TpsDig software (Rohlf 2005).

Females have significantly larger skull (both ventral and dorsal side) than males in both species. Males and females do not differ significantly between species in terms of centroid sizes (CS). Shape analysis revealed a non-significant shape difference between sexes and between species. The sex and species interactions were not significant for both sides of the skull as well. Significant species x CS and sex x CS interactions in dorsal cranium indicate that size-dependent shape changes differ between species and sexes. Besides, a marginally significant species x sex and sex x CS interactions were found in ventral cranium.

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Comparison between the skin structure of the slow worm (*Anguis fragilis*) and sand lizard (*Lacerta agilis*)

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The stratifying, multilayered epidermis of all vertebrate species creates the outermost part of the skin, that forms a barrier protecting the body from dehydration, mechanical trauma and microbial intrusion. Many taxa developed additional bony dermal structures supporting the upper layer of skin. These are called osteoderms and has been found in: amphibians, squamate reptiles, turtles, crocodiles, dinosaurs and mammals. Osteoderms are responsible for many different functions such as: thermoregulation, various forms of defense and blood pH regulation.

Our study considers two species of squamate reptiles: one non-armored (sand lizard *Lacerta agilis*) and one armored lizard (slow worm *Anguis fragilis*) in which osteoderms cover most of their body. These two species represent two different large families of Squamata (Lacertidae vs. Anguidae) and pursuing different lifestyles (terrestrial vs. semi-fossorial). The purpose of this study is to determine if presence of osteoderms has any impact on skin structure and ultrastructure. We described the structure of skin in this two species on the level of light, scanning and transmission electron microscopy.

Approvals for research were obtained from the Local Ethics Commission in Katowice (41/2010; 87/2015), the General Directorate for Environmental Protection of the Polish Ministry of Environment (ref. No: DOPOzgiz-4200/II-88/4189/10/JRO) and the Regional Directorate for Environmental Protection in Katowice (WPN.6401.257.2015.DC).

Comparative analysis of reptilian epidermal differentiation genes reveals similarities between proteins in turtle scutes and human skin barrier proteins

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The evolution of the skin in terrestrial vertebrates was facilitated by the evolution of specific structural proteins. Many of these proteins are encoded by genes clustered in the so-called epidermal differentiation complex (EDC) which has originated in a common ancestor of mammals and sauropsids.

Here we investigated the hypothesis that the evolution of mechanically resilient modifications of the reptilian epidermis might have been associated with specific adaptations of the EDC. By comparative genomics, de novo gene predictions and experimental confirmation of gene expression, we show that the EDCs of sauropsids comprise approximately 100 genes that are specifically expressed in the skin. Notably, corneous beta-proteins previously known as beta-keratins are also encoded at this locus in sauropsids. Several EDC proteins have evolved cysteine/proline contents beyond 50% of total amino acid residues and contain sequence motifs also present in mammalian small proline-rich proteins (SPRRs). In turtles distinct subfamilies of EDC genes have been expanded and partly translocated to loci outside of the EDC. RT-PCR analysis showed that EDC genes are differentially expressed in the skin of the various body sites and that a subset of beta-protein genes both within the EDC and those located outside of the EDC are expressed predominantly in the scutes of the turtle shell.

Our findings support the hypothesis that the evolutionary innovation of the turtle shell involved specific molecular adaptations of epidermal differentiation. Furthermore, we provide evidence that several amino acid sequence features of the EDC-encoded proteins have been conserved in amniotes ranging from turtles to humans.

Testing sexual selection in an explosive amphibian breeder (*Bufo bufo* LINNAEUS 1758)

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Male strategy in anurans depends on intensity of intra-sexual (competition between males) versus inter-sexual competition (attracting females) (Wheeler & Welsh Jr 2008). Adequate body size often enables advantage over opponents (Arntzen 1999), while certain skills, not necessarily correlated with body size, offer higher attractiveness to opposite sex (Vitt & Caldwell 2009).

A pilot analysis performed in 2005 on reproductive males in one population of Common Toad (*Bufo bufo*) tested variation in their body length, body mass, fore and hind limb length and revealed absence of differences in body weight or body length between two groups of males of different mating status – those in amplexus and solitary ones (Man Whitney U test for nonparametric statistics, nonsignificant p values). However, males in amplexus had longer forelimb than single males ($Z=-2.93$, $p<0.01$).

Following the same study design we measured male toads of both reproductive status from the same local population during breeding seasons in 2011, 2013, 2014 and 2015 year. In pooled samples solitary males and males in amplexus differed only by body length (males in amplexus were bigger: $Z=-4.15$, $p<0.0001$). Spearman rank-correlation showed that body length of unpaired males decreased with years (Spearman $R=-0.20$, $p=0.003$) while in paired males group it did not change (Spearman $R=-0.06$, $p=0.44$). Although both the effective population size and the number of males per female at the peak of reproductive season insignificantly varied over the study period, there is inclination toward decrease of the first and increase of the second parameter with time.

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Head features as a tool for squamate embryo's age evaluation

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The evaluation of the embryo age is great challenge for studies concerning developmental biology. Tables of developmental stages are indispensable to any experimental study of embryos in almost all vertebrate species. In reptiles, as ectotherm animals, development is strongly dependent on environmental factors, mainly temperature which makes developmental tables even more important. Within the many sets of characters its difficult to find the one which would enable accurate evaluation of embryo age and at the same time would be useful in comparative studies. The appropriate set of features should be relatively conservative and causal. Features of the head seem to fulfill both criteria. Moreover their developmental sequences proved to be useful tool in phylogenetical inferences.

We used embryos of sand lizard *Lacerta agilis* as a model species for this study. The sand lizard is a common lizard species in Europe and Western Asia. Morphological description was based on the analysis of embryonic developmental characters examined under stereo microscope. The list of diagnostic characteristics included scales pattern, scales pigmentation, shape, cerebro - and viscerocranium appearance, the degree of eyes development: lateral and parietal, level of medial nasal, lateral nasal and maxillary processes connection, eyelids development, jaw development, patency of external naris.

Approvals for research were obtained from the Local Ethics Commission in Katowice (41/2010; 87/2015), the General Directorate for Environmental Protection of the Polish Ministry of Environment (ref. No: DOPozgiz-4200/II-88/4189/10/JRO) and the Regional Directorate for Environmental Protection in Katowice (WPN.6401.257.2015.DC).

Age and sex structure among three newts species in the Tłumaczów quarry in southern Poland

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Demographic data of populations are an important tool to support the management of amphibian populations. In a human-dominated landscape artificial breeding sites such as quarries seem an important habitat for biodiversity, especially after the end of opencast exploitation.

The aim of our research was to compare age and sex structure within three newts species (*Ichthyosaura alpestris*, *Lissotriton vulgaris*, *Triturus cristatus*) in the Tłumaczów quarry of melaphyre in southern Poland. We used a drift fence to collect animals (N=2538) during the spring migration from 21.03 to 29.04.2012, and used skeletochronology to estimate the age structure of a subset of randomly selected individuals.

Age assessment between and within observers overlapped (Spearman's rho, $r = 0.874$, $p < 0.05$ and $r = 0.972$, $p < 0.05$). The adult age of *I. alpestris* ranged from 3+ to 10+ years in males (N=43; avg. age 5.95 ± 1.61), and from 4+ to 10+ years in females (N=35; avg. age 6.57 ± 1.48).

The age of breeding males of *L. vulgaris* ranged from 3+ to 10+ years (N=21; avg. age 5.57 ± 1.31), and from 4+ to 10+ years in females (N=28; avg. age 5.32 ± 1.61).

Breeding males of *T. cristatus* ranged from 3+ to 7+ years (N=33; avg. age 5.30 ± 1.47), and from 3+ to 9+ years in females (N=35; avg. age 5.42 ± 1.92). We did not find significant differences in age structure between the sexes in any of the studied species.

Based on the available data we suggest that this newts populations are the largest in the south-west Poland. This site is unique and should be protected by law.

Are size sexual dimorphism and sexual determination mode evolutionarily associated in geckoes?

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Sexual differentiation in bisexual organisms results from differences in reproductive roles of the two genders triggered by anisogamy and one of its most remarkable outcomes is sexual size dimorphism (SSD). In turn, it has been suggested that SSD is at the basis of the evolution of sexual chromosomes, with important consequences for sex determination mode. Geckoes are an excellent model for studying the coevolution of SSD and sex determination mode. They include both male- and female-biased SSD species, and they encompass various cases of temperature-dependent (TSD), as well as genetic determination (GSD), including both types of chromosomal systems (XY and ZW).

We tested how SSD varies among different sex determination modes in a macroevolutionary framework, based on a dataset including about 40 gecko species. Our results indicate a strong association between both traits, but this is largely driven by shared evolutionary history. The magnitude of SSD did not differ between TSD and GSD species, not supporting the hypothesis of the resolution of sexual conflict through the evolution of sexual chromosomes. Contrary to our predictions, TSD species exhibited male-biased SSD, independently of the male or female incubation temperature regimes, and ZW species were close to monomorphic, while XY species tended towards female-biased SSD.

Our results challenge the relevance of SSD for the evolution of sex chromosomes, and oppose models that explain variation in SSD in ectotherms based on incubation temperatures, suggesting that other mechanisms, such as sexual selection and differential post-natal growth, are probably more important in shaping macroevolutionary patterns of SSD.

Do it yourself: using open-source hardware and low-cost sensors in reptilian thermal biology

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While open-source software is by now an established concept, open-source hardware is still underused or even ignored. Defined as “*hardware whose design is made publicly available so that anyone can study, modify, distribute, make, and sell the design or hardware based on that design*” (OSHW 2017), open-hardware was fundamentally developed and advanced by the global “maker” movement during the last 15 years. The design of open-source scientific instrumentation has been an integral part of this growth, leading to impressive results on the academic level as well (e.g. the launch of HardwareX, a dedicated Elsevier journal in 2017).

Here we present two different approaches to construct a low cost temperature data logger using the popular Arduino platform and affordable digital sensors, which can be deployed to accurately assess operative temperatures in reptilian thermal biology studies (Hertz et al 1993; Bakken & Angilletta 2014; Vickers & Schwarzkopf 2016). First we present a modular design that is based on an existing project from the field of physical geography (Cave Pearl Project: Mallon & Beddows 2014, 2015). We also present an open-source circuit board that we purposely designed, which achieves the same functionality. Both designs are low cost, low power and demand little coding and technical skills. Finally, we assessed the impact of sensor accuracy on operative temperatures and results suggest that $\pm 0.1^{\circ}\text{C}$ and $\pm 0.5^{\circ}\text{C}$ accuracies produce similar operative temperatures, thus removing the need for further calibration from the user.

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High Genetic Differentiation and Limited Gene Flow among Bavarian Adders (*Vipera berus* complex)

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In the Alpine region, a mitochondrially distinct clade of *Vipera berus*, the “Alpine form” meets the nominate form, *V. berus berus* (Kalyabina-Hauf et al., 2004, Ursenbacher et al. 2006). We studied Southern Bavarian adder populations using microsatellite patterns to confirm these clades and to assess if there was gene flow between them.

The microsatellite spectra confirm the existence of two main population groups in Southern Bavaria, but a third group in Austria is also shown (preliminary data). Actual gene flow between the groups is low but it may have occurred to a greater extent in the past. There is no perfect congruence between mitochondrial and microsatellite based groups, indicating asymmetric gene flow, probably sex-based (higher mobility of males).

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Digitized type specimens collection of amphibians and reptiles in the Zoological Institute of the Russian Academy of Sciences, St. Petersburg

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An information retrieval system for type specimens stored in research collections of the Zoological Institute, Russian Academy of Sciences was created in 2015. Its conceptual structure is built on «client-server» technology and includes: 1) the data layer («server») is a taxonomic classifier in the ZOOCOD 4 standard, specimens catalogues, geographical classifiers, supporting and linked dictionaries, tables, hierarchical thesauri, etc.; 2) the presentation layer («client side») – set of user interfaces of the information retrieval system for public access via the Internet. Improving functions and expansion of the taxonomic classifier of animals «Animalia» was conducted (<http://www.zin.ru/zoodiv/animals.asp>).

In the taxonomic tree of the classifier for taxa of the family level and below, information about type specimens availability in research collections of the Zoological Institute of the Russian Academy of Sciences is displayed with direct links to individual specimen's pages on the website for digitized collections (<https://www.zin.ru/collections/>).

The collection catalog is technically implemented within the environment of Microsoft SQL Server 2016 and includes more than 70 primary and auxiliary tables with more than 220 specialized elements of SQL programming.

118 type specimens of amphibians of 28 taxa were digitized with 705 high-resolution graphic images and presented on the website of the Zoological Institute, St. Petersburg (<https://www.zin.ru/collections/Amphibia/>).

Digitization of 157 type specimens of reptiles (149 recent and 9 fossils) belonging to 41 taxa was made with presentation of 874 high-resolution graphic images on the website of the Zoological Institute, St. Petersburg (<https://www.zin.ru/collections/Reptilia/>).

Microscale habitat preferences and habitat use of *Coronella austriaca* – a geoinformatic approach

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Based on digital elevation models, high-resolution orthophotomap, meteorological and population data collected in 2016 in the post-mining area in Opole Voivodeship, Poland, we checked the impact of microscale habitat variables on the distribution of the smooth snake *Coronella austriaca*.

The following methods were used: image supervised classification, focal statistics, density function, map algebra, stochastic and deterministic interpolation methods, spatial autocorrelation and spatial distribution analysis. Environmental variables were obtained and subsequently used in logistic regression and multi-variate regression analysis. Results show that land cover significantly affects the microscale distribution of smooth snakes. Greater share of bare soil positively affects the probability of the snake's occurrence, while conifer cover had negative effect. Unexpectedly, no relationship between microclimatic factors and occurrence of snakes was found, which requires further analysis. The snakes were found on 4-41°C, measured on 5 cm above ground level.

There was a spatial diversification between young and adult individuals, which may result from intraspecific predation. There was no difference between the land cover preferences between two age groups – all snakes prefer ecotonic zones, with a high share (40-50%) of bare soil but also covered by some trees, shrubs and grass. Young individuals occupied sites with higher average vegetation height and lower potential insolation, which may suggest that they choose hiding rather than thermoregulation. There was a difference in the size of occupied area between age groups – young individuals occupied larger areas than adults.

The results should be taken into consideration in planning the species' active protection and modify existing regulations.

Type locality of *Holodactylus africanus*

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Oskar Boettger described *Holodactylus africanus* from only one specimen collected in Abdallah to the north of Webi (Webi means River in Somali language) in 1893 (Boettger 1893). Unfortunately, it is impossible to find the locality Abdallah in present maps. The type specimen was collected during the expedition of prince Eugenio Ruspoli in 1891 which went south from Berbera to Shebelle River (Ruspoli 1892, Milosevich 1895). It was found out by comparison and study of period literature and maps, that Abdallah was the name of tribe living in area of Shebelle River (Rossi 1898).

Expedition of prince Ruspoli started in Berbera (10°26' N, 45°00' E) and walked through Golis Mountains, Oodweyne (9°24' N, 45°04' E) to Warandab (7°08' N, 44°07' E) where they reached the Fafen River. Then the expedition continued along the Fafen River to the south to Shebelle River. After crossing of the Shebelle River, the expedition vain attempted to reach the Juba River, but they only travelled to the south alongside Shebelle River. They returned finally to the Shebelle River near Imi (6°28' N, 42°08' E), where the river change the watercourse from south to east.

In this region the place named Hemlo, occupied by Abdallah tribe, was mentioned in travelogue of Emilio Dal Seno (Rossi 1898). On the way back the Expedition set out north-eastern direction to Warandab and then to north to Berbera.

The important part of the research is also the map of the route of the first expedition of prince Eugenio Ruspoli in 1893.

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Body size in highland populations of *Testudo hermanni* and *Testudo marginata* in the Basin of Feneos (Chelmos Mountains, Peloponnese, Greece)

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Small body size and dwarfism have been suggested as a morphological response to hot, dry and poor habitat conditions in *Testudo* species occurring in lowland habitats of the Peloponnese peninsula (Fritz et al. 2005). Up to now, little is known about body size of *Testudo* populations in comparative humid habitats in the highlands of the southern Balkans.

Therefore, we measured straight carapace length (SCL) and body weight of 153 *Testudo marginata* and 559 *Testudo hermanni* in the Basin of Feneos (800-1100 m a.s.l.). Our results showed an average SCL of 19,1 cm \pm 1,7 in females / 16,1 cm \pm 1,4 in males of *Testudo hermanni*, 28,9 cm \pm 3,2 in females / 30,4 cm \pm 2,5 in males of *Testudo marginata* and an average weight of 1,3 kg \pm 0,4 in females / 0,8 kg \pm 0,2 in males of *T. hermanni* and 3,4 kg \pm 0,7 in females / 3,2 kg \pm 0,8 kg in males of *T. marginata* within this population. Body size peak values of *Testudo marginata* exceeded a SCL of 42,00 cm and a weight of 6 kg, which have never been documented at the Peloponnese before.

Compared with data of lowland populations given in Artner (1996), Bour (1996), Artner (1997) and Willemsen & Hailey (1999), our data suggest larger average body size in *Testudo* populations occurring in the moist and cooler Peloponnese highlands than in lowland populations.

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Perils of ingesting harmful prey by macrostomatan snakes

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Snakes are able to immobilize, ingest, transport, and swallow prey of relatively large size as well as dangerous items such as other predators, hard-biting, spiny, or toxic prey. Various incidents have been reported of snakes failing to complete the prey consumption process and being injured or killed during different phases of feeding.

Here we provide the first extensive review of such incidents, including 45 publications describing over 68 mortality cases caused by ingestion or attempted consumption of injurious prey. We also report 12 previously unpublished cases from the USA and Bulgaria, including mortality of five juvenile piscivorous snakes (*Natrix tessellata*) from one location. Overall, we identified at least 41 species representing 6 families and 29 genera. We discuss different feeding scenarios that can lead to negative or even fatal impacts of prey on predatory snakes, and we classify such incidents into four major categories. We discuss a special case of the highly toxic and invasive Cane toad (*Rhinella marina*) in Australia and other locations in which it has been introduced. We perform a meta-analysis of the characteristics of the accumulated data in an attempt to elucidate overarching causes that led to the snakes' mortality. We conclude the review with a set of general conclusions and propose ideas for further research.

New Red List of the Czech Republic Herpetofauna

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Red list of amphibians and reptiles of the Czech Republic was last time prepared at 2003 so there was need for updating it (Jeřábková et al. 2017). Evaluation of species status followed IUCN guidelines and was based on extensive data collection starting at 2008. There were two all-country wide surveys and several smaller ones which brought us data about species areas, population size and trends. This time all country was evaluated as one unit regardless existing differences between Bohemia (west) and Moravia (east part of the CR).

RL status of the majority species did not change since 2003, however there are several changes, mostly increase. There was status decrease in 3 species only (from CR to EN in all cases). In the case of *Triturus carnifex* more localities were found and some populations are doing better. There are numerous Moravian populations of *Lacerta viridis* so despite decrease in many Bohemian populations it cannot be evaluated as CR. Third species is *Zamenis longissimus*. In its case is improvement caused by implementation of Action plan. The most important was stabilization of most isolated and endangered population around upper Ohře river.

There are ten species which status worsened. In case of *Bufo viridis* and *Epidalea calamita* many localities vanish due to extensive changes in land use. But also “common” species are in this group as *Bufo bufo*, *Rana temporaria*, *Lacerta agilis* and *Natrix natrix*. There is obvious decrease in population density in their case even they are not really rare yet.

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First survey of pathogenic fungus *Batrachochytrium salamandrivorans* in wild and captive amphibians in the Czech Republic

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Batrachochytrium salamandrivorans (*Bsal*) is a recently recognized parasitic fungus infecting newts and salamanders (Spitzen-van der Sluijs et al. 2013, Martel et al. 2013). It is lethal to many European caudata species. Surveillance of this pathogen is of great conservation importance (Council of Europe 2015). As the pet trade is assumed to be the route of *Bsal* transmission (Martel et al. 2014), it is likely that large cities where exotic pet collections, specialized pet shops and zoos are more common are at greater risk of *Bsal* emergence.

Therefore, we have started to survey wild populations of Caudata species (3 species, 9 localities, 126 samples), as well as collections of captive salamanders (60 species, 198 samples) in Prague, the capital city of the Czech Republic, and its surroundings. Sampling and DNA extraction were performed according to procedures used in amphibian chytridiomycosis research (Boyle et al. 2004). *Bsal* presence was analyzed by qPCR (Bloom et al. 2013). *Bsal* was not detected in any of the 324 tested samples. Because the risk for *Bsal* to spread in Europe via the pet trade is ever-present (Spitzen-van der Sluijs et al. 2016), additional steps to alleviate such risk should be undertaken especially in proximity to larger cities.

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Post-breeding migration and habitat use of *Bufo bufo* in a vineyard-dominated landscape

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Habitat destruction due to an intensification of farming and a concordant increase of pesticide applications are named as two of the most important factors for the global amphibian decline. The knowledge of how amphibians are using the agricultural landscape and how pesticides might affect amphibians is of essential importance for conservation efforts.

Since mid-March 2017 we are applying radiotelemetry to investigate the habitat use and post-breeding migration of 20 adult common toads (*Bufo bufo*) from one breeding pond in a vineyard-dominated landscape near Landau in der Pfalz (Germany). Based on long-term data from an amphibian fence in a distance of about 500 m to the breeding pond, we expected that most individuals would migrate from the near Palatinate Forest (distance 1 km) to the pond in spring and return to the forest after mating, to use it as terrestrial habitat. We located the toads on a daily basis, where every movement over 30 cm was recorded, and described the occupied habitat.

Our preliminary data shows that after 2.5 months post mating, only one individual has reached the forest. The remaining toads are staying in the agricultural landscape, where they are primarily using slopes and meadows as habitat. This shows how important a richly structured landscape is for amphibians. Nevertheless, we located some individuals directly in the vineyards, confirming a potential exposure to pesticides. Besides the description of the post-breeding movements and the used habitats we discuss the spatial-temporal overlap of pesticides with the presence of *B. bufo* in the vineyards.

Isolated populations of *Salamandra s. salamandra* in Alpine areas of Salzburg

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In the state of Salzburg, the Fire Salamander (*Salamandra s. salamandra*) can be mainly found in hilly areas of the north between 400 and 800 m a.s.l. The southern borders of the continuous distribution range is located in the Calcareous Alps close to Lofer and Golling. Some isolated populations have been reported from the central Alps but have not been evaluated for decades.

A cooperation project of the Herpetological Study Group, Haus der Natur, the Austrian Society of Herpetology (ÖGH) and the Austrian League for Nature Conservation (ÖNB), sponsored by the companies Spitz and Hofer KG aimed at improve the knowledge on distribution and habitats of Alpine populations of Fire salamanders, evaluate the status of old records, map the current habitat situation and make suggestions for habitat enhancement measures, when needed.

All available data were consolidated. Seven 5*5 km study areas were defined, that contain old records or suitable habitat. Between June and August 2016 and again in April 2017, five to seven brook systems in each study area were checked by day for the occurrence of larvae of *S. salamandra*. Simultaneously, calls for reports of Salamander sightings were communicated via local media and the citizen science platform www.naturbeobachtung.at.

In only two out of seven study areas we could observe *S. salamandra* or get reliable evidence from locals. Two new records could be registered by citizen science results.

Amphibians of the Amazonian floating meadows: zeta-diversity, processes, and patterns

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The Amazon catchment is the largest river basin and contains the most extensive rainforests of the world. At least 30% of its waters flow across floodplains. Floating meadows are extensive plant mats typical of these flooded areas. Besides playing an important role in the nutrient cycles, this floating vegetation also provides shelter, feeding, reproduction and nursery habitats for many kinds of organisms.

Using our own data collected in the field (36 localities) and from the literature (13 localities), we investigated amphibian diversity in floating meadows in the Amazon Basin. To this end, we used zeta diversity (i.e. number of species shared by multiple assemblages), a new concept and metric that unifies incidence-based diversity measures, patterns, and relationships.

Considering the 49 localities, we found 45 anuran species, of which 80% were hylids. In average, our analyses suggested that there are 10 species in one sample (alpha diversity), 5 species shared by any two samples (pairwise similarity) and 3 species shared by any three samples. Our analyses further revealed that one species is shared by any five, six or seven localities. From any eight localities on, our analyses suggest that no species are shared. The community assemblages are driven by both neutral and niche-based processes (e.g. habitat selection), with common species governed by neutral forces and rare species by niche-based forces.

“Experience, report and protect reptiles” - A citizen science project in Austria

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Active nature observation is a good possibility to get people enthusiastic about nature and motivate them to protect it. www.naturbeobachtung.at is Austria's oldest online-platform to report nature observations with more than 340.000 observations and 197.000 pictures uploaded, containing a network of about 5.600 citizen scientists, 40 scientific experts and platform operators.

In this project, the Austrian League for Nature Conservation (Naturschutzbund Österreich) and partners invite the public, especially in the county of Styria, to experience the 14 different reptile species of Austria, all of them endangered, during excursions and lectures. Thereafter people are asked to report their own reptile observations on the website www.naturbeobachtung.at.

Up to now, there are about 1.970 reptile observations reported on the platform, 60% of them with picture. The reported observations are constantly validated by experts through using a special system on the website to verify the uploaded pictures. Moreover, the project wants to increase sympathy for this rather unpopular group of animals by carrying out intense public relations and distributing flyers, field guides and posters. It also approaches local communities and schools. Our partner “Berg- und Naturwacht Steiermark” is in charge of snake emergencies in Styria and also reports these data on the website. All reptile observation data are displayed in interactive distribution maps as well as in different image galleries and will be the basis for field mapping and protection measures on selected areas in the third year of the project.

On distribution and origin of allochthonous common wall lizard (*Podarcis muralis*) populations along the Railroad tracks in the province of Salzburg

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Podarcis muralis is a small Mediterranean lizard, which is native to large parts of middle and southern Europe, and also in parts of Austria, but not in the province of Salzburg. In 2008 the first allochthonous wall lizard population in the province of Salzburg has been discovered at the railway station of Steindorf/Straßwalchen. In the years 2014 and 2015, two other populations, in the city of Salzburg and in Schwarzach/St. Veit, have been found by members of the herpetological work group of the Haus der Natur.

The objective of this work is to ascertain the overall distribution and origin of this species in Salzburg and furthermore trying to establish a model of the species dispersal over the next decade. Study sites were chosen based on the railroad net of Salzburg and areas that are favoured by warm climate. The species distribution is assessed by specific search of appropriate structures in the surroundings of railways. DNA sampling is performed with buccal swabs. Since all three known populations, plus a newly discovered one in the adjacent region of Upper Austria, have been found either in the immediate surroundings, or directly at railroad tracks, it is assumed that the species was introduced through transportation of cargo – most probably through the transportation of natural stones, such as marble. First results of the DNA sampling indicate northern Italy as the place of origin.

Phylogeography of the yellow-bellied toad (*Bombina variegata*), reveals additional refugium in Central Greece

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In this study, we used fragments of 1096bp of the mitochondrial Cytb gene to supplement the existing knowledge on the phylogeographic structure of *Bombina variegata* at the southern limit of species distribution, with the implementation of additional samples from Greece.

Using Maximum Likelihood and Bayesian Inference methods, a total of 14 new haplotypes from Greece were found, attributed in three clades. Northeastern Greek samples grouped within the formerly described Rhodopean Clade (Hofman et al. 2007, Fijarczyk et al. 2011), which consists of samples from Rhodope Mts, and Bulgaria. A large number of haplotypes are grouped within Clade BW3 which is spread over an extensive area towards the west, from Greece to the forelands of the Alps (Hofman et al. 2007, Fijarczyk et al. 2011). However, two haplotypes from Central Greece were found to constitute a new Clade (BW4) that exhibits considerable differentiation with the above mentioned Clades (% p-distance from 1.4% to 2.6%).

The existence of the three haplotype groups within a small geographic area (Greece), coincides with the “refugia-within-refugia” hypothesis for the Balkan Peninsula, as has been proposed for other amphibian and reptile species and outlines the contribution of peripheral populations in the intraspecific genetic variation. Further research is needed in order to define the exact geographic distribution of the new clade, while such information should be considered in future conservation and management actions.

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Amphibians and people share the benefits of sustainable urban drainage (SuDS) ponds in a fast-developing city

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Sustainable Drainage Systems (SuDS) manage storm-water, reducing flood risk and diffuse pollution, through processes mimicking nature (Woods-Ballard et al. 2015). We postulated that SuDS might bring three benefits to biodiversity (O'Brien 2015): as breeding sites; by connecting otherwise isolated populations to form metapopulations within a habitat network; and by bringing urban-dwellers into contact with nature. Amphibians were chosen as a model taxon due to their relative ease of detectability, limited dispersal and pollution sensitivity.

This eight-year study in Inverness, UK, found that amphibians bred successfully in 21/23 SuDS ponds, and that SuDS have characteristics associated with high quality habitat. Different measures of SuDS ecological quality were highly correlated. Unlike previous studies of urban amphibians (e.g. Hitchings & Beebee 1998), we found no reduction in genotypic diversity compared to those from countryside ponds. We held a public event at one of the ponds, attracting over 100 people, mainly local families with children, suggesting that, when made aware of their value, people are genuinely enthusiastic. For children, growing up in contact with nature has been linked to improved mental health and educational outcomes (Bingley & Milligan 2004). However, we found that these benefits are not equitably distributed: poorer neighbourhoods have SuDS of lower ecological quality, though this can be improved by design and management.

In this fast-growing city, SuDS are generating multiple benefits for amphibians and humans, whilst also serving an important role in the drainage system, and these could be further increased through improved design and management.

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The Population Genetic Structuring of Three *Heremites* (Gray, 1845) (Reptilia: Scincidae) Species in Anatolia

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The phylogeny and genetic structures of the Anatolian populations of the genus *Heremites* were investigated by using a total of 2856 bp comprising two mitochondrial (COI and cytB) and two nuclear (c-MOS and MC1R) gene fragments. Analyses were performed on 128 specimens of 26 populations, representing three species (*H. auratus*, *H. septemtaeniatus* and *H. vittatus*).

The haplotype and nucleotide diversity indices were found to be high for all species. The recovered phylogenetic tree has two main clades under Bayesian Inference and Maximum Likelihood methods: *H. auratus*, *H. septemtaeniatus* and *H. vittatus* gaining high support values. The all haplotypes of *H. septemtaeniatus* formed a single cluster, while the haplotypes of *H. auratus* and *H. vittatus* grouped in a few subclades corresponding to their geographical distribution. The fine-scale genetic structuring observed in network analysis was also largely consistent with the phylogeny. The results indicate that the geographical and paleoclimatic history and topography of Anatolia had played important roles in diversification of the genus.

Financial support of this study was provided by TÜBİTAK via a research project with grant number 113Z752.

Distribution range of *Bufo verrucosissimus* (Pallas, 1814) along the Black Sea coast of Turkey inferred from cytochrome b region

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The common toads of western palearctic consist of four species; *Bufo bufo*, *Bufo eichwaldi*, *Bufo spinosus* and *Bufo verrucosissimus*. *Bufo bufo* is widespread in Europe and is found through much of Turkey. *Bufo verrucosissimus* inhabits the Caucasus and northeastern Turkey, but the distribution range is still unknown.

We studied 36 individuals from 12 different localities between Kırklareli and Artvin in Turkey. We used DNA sequence analyses on 796 bp of the mitochondrial cytochrome b region to resolve how far *B. verrucosissimus* distributed in the Black Sea Region. Haplotype networks were constructed with PopART 1.7. Maximum Likelihood and Bayesian analyses were applied including Caucasian *B. verrucosissimus* and European *B. bufo* sequences from GenBank.

Our results show that all the studied individuals in Turkey were in the same clade with European *B. bufo* except Artvin individuals. In conclusion, *B. verrucosissimus* inhabits only Artvin city (near Georgia) when considering the whole Black Sea and Marmara Region. Therefore, the distribution range of *B. verrucosissimus* extends into Rize along the Black Sea coast and contact zone with *B. verrucosissimus* and *B. bufo* is probably between Artvin and Rize in Turkey.

This study was supported by TÜBİTAK under project number 114Z823.

Feeding ecology of *Pelophylax cerigensis*, the most endangered frog in Europe

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The Karpathos Marsh frog (*Pelophylax cerigensis*) is endemic to the island of Karpathos (Aegean Sea, Greece). It is considered the most endangered amphibian in Europe (Temple & Cox 2009) because of its restricted range that comprises only two small rivers at the north part of the island. Since the first description of the species (Beerli et al. 1994) there has been no further information published on its biology.

Here we assess for the first time the feeding ecology of the species, one of the most important parameters on amphibian biology. We examined a total of 68 individuals with the stomach flushing method (Solé et al. 2005) and also measured body weight, snout-vent length and mouth width. We also recorded width and length for prey items. Prey availability was evaluated with the quadrat counts method.

Pelophylax cerigensis adopted the general feeding pattern of other green frogs of the adjacent areas where beetles, spiders, isopods and gastropods represent the main prey groups (Çiçek 2011, Bisa et al. 2007, Plitsi et al. 2016). Though we found a comparative preference for spiders and beetles, we failed to detect any incidents of avoidance of specific prey groups. It seems that *P. cerigensis* follows an opportunistic feeding strategy, consuming what its poor environment can provide and manages to survive in small modicum.

This study was sponsored by the Mohamed bin Zayed Species Conservation Fund.

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Navigation in the rainforest: experience based homing in poison frogs

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Animal navigation has become one of the most comparative fields in behavioral biology. However, orientation under natural conditions has been mostly studied in animals moving in open spaces, such as birds and honeybees in flight, ants and amphibians in open field, or sea-turtles and fish in open water. Little is known about how animals find their way in the understory of the rainforest, where many cues, such as distant landmarks are limited.

Poison frogs (Dendrobatidae) are small inhabitants of the Neotropical rain forest understory and they show some of the most complex spatial behaviors among amphibians, such as long distance tadpole transport. We studied the homing behavior of two territorial poison frog species in the field using telemetry. We revealed that males of *Ameerega trivittata* return to their home territory via a direct path after experimental translocations of up to 800m. We also found that a small dendrobatid frog *Allobates femoralis* relies on experience to find the way home from up to 400m. Taken together, our results suggest that poison frogs form a spatial map of a large area but the exact landmarks being used remain unknown.

Temporal variation and effect of climatic conditions on spring migrations of amphibians to reproduction sites in Ljubljana, Slovenia

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Most amphibians require terrestrial habitats for foraging and aquatic habitats for development of eggs and larvae. Mass migrations of several amphibian species occur in early spring, when they travel from winter quarters to reproduction sites. Their migration paths are often crossed by roads and road mortality of amphibians on such road sections can be very high. One of such identified road sections with high amphibian mortality is located in the capital of Slovenia, Ljubljana. The Slovene Herpetological society – *Societas herpetologica slovenica* has been setting up a temporary amphibian fence in spring for the past nine years. Along with informing the general public about the importance of conservation and safe keeping of amphibians, data about the number, species composition and the exact location of amphibians crossing the roads was collected.

Migrating species are primarily common toads (*Bufo bufo*), common frogs (*Rana temporaria*) and agile frogs (*R. dalmatina*). The migration period usually begins in late February or early March and lasts only a few weeks. The aim of this work was to analyse the temporal variation of amphibian migrations and how the temperature and precipitation affects the migration of each species and their numbers, using the data from in the past four years (2014 - 2017). Altogether, more than 10.741 individual amphibians were carried over the road in this period. Inter-annual comparison reveals a variable temporal pattern of amphibian migrations with temperature as a more influential factor than precipitation for the start of migrations.

Amphibian communities of the Mátra Mountains (Hungary)

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The Mátra is part of the North Hungarian Mountains and belongs by origin to the largest young volcanic zone of Europe. It is known, that this mountain range has a rich and varied amphibian fauna, but we know less about the breeding communities and the co-occurrence of the different species. We aimed to assess the relative contribution of local variables and spatial factors on the assembly of the amphibian communities.

We conducted our study between April and June 2017 in different parts of the Mátra Mountains and we found amphibian species in 68 different waterbodies. We included ponds, temporary pools and streams in our survey. Hand netting supplemented by visual searching was used to record amphibians and dip netting for larvae and newts. Water temperature, area of the waterbody, water depth and vegetation cover were determined on the field. In RStudio the 'cooccur' package was used for the co-occurrence analysis. The waterbodies exhibited a wide range of water depth along with a wide range of vegetation cover. We detected 11 amphibian taxa in the study area. The yellow-bellied toad (*Bombina variegata*) was the most abundant species, which occurred mainly alone in small temporary pools on forest roads. The fire-bellied toad (*Bombina bombina*) is a typical lowland species in Hungary, but in the Mátra it occurs at relatively high elevations (up to 600 m). Most species were found in bigger ponds with higher vegetation cover. Conserving a high diversity of aquatic habitats are essential for the long-term protection of diverse amphibian communities.

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Relevance of new natural history insights on conservation planning for a once lost frog

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Despite the fact that the recently rediscovered Hula painted frog (*Latonia nigriventer*) has lately been identified as the sole relict of a clade that was thought to have gone extinct during the Pleistocene, this species has remained one of the rarest and most poorly understood amphibians worldwide.

In order to be able to formulate precise conservation strategies, we gathered data on multiple aspects of the Hula painted frog's natural history. Our surveys confirmed that *L. nigriventer* is a localised species with elusive habits. The species appears to follow an opportunistic breeding phenology and has a tadpole morphology similar to its well-studied sister group *Discoglossus*. However, the adults' extended annual presence in the aquatic habitat is a major difference from species of *Discoglossus* that mostly breed in ephemeral water bodies.

The revelations that *L. nigriventer* exhibits a relatively low dispersal ability and strongly depends on aquatic habitats, essentially guide future conservation planning. To date, most of the canals within its distribution range are only aquiferous during the rainy season when the species appears to be less active. Newly created permanent canals in close vicinity to each other could offer this ancient frog species more suitable alternatives and may even enhance dispersal.

Limb amputations and malformations of the endangered *Alytes muletensis*. Are they true threats for adults?

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Limb malformations and developmental abnormalities in natural populations of amphibians have been reported for decades but few reports involve deformities in rare and endangered amphibians. The Majorcan midwife toad, *Alytes muletensis*, is an endemic and endangered species from the island of Mallorca (Balearic Islands, Spain). Nearly three decades of conservation efforts have guaranteed the preservation of this amphibian species. The introduction of invasive species such as *Natrix maura* and *Batrachochytrium dendrobatidis* are meant to be the main threats, although not the only ones.

Here is presented a compilation of information related to limb amputations and limb malformations recorded over the last twelve years of adult monitoring. Limb malformations in *A. muletensis* were previously reported on a single natural population showing a malformation rate of 5.45 % but during the last years malformations have been recorded in new populations. On the other fore limb amputations have been documented as relatively frequent in males due to the reproductive activity, but new information shows that fore and hind limbs amputations are more frequent than the expected resulting a possible conservation problem in some populations.

Effects of food availability and photoperiod on body size and growth rate of the fire salamander (*Salamandra salamandra*) larvae

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A major goal of life history theory is to explain and predict how variation in growth and body size of organisms occurs. Food resource limitation and photoperiod are important factors that affect these life history traits in organisms living in seasonal environments. Many studies have focused on phenotypic plasticity of larval amphibian but few experimental studies have considered the joint effects of food and photoperiod constraints.

We studied such interaction effects on body size (total length and body mass), growth rate and time to metamorphosis, by manipulating food resource level (high vs. low food) and photoperiod conditions (zero vs. eight hours of light) in the larvae of the fire salamander, *Salamandra salamandra*, in 140 days window of time.

The results indicated that life history responses to food and photoperiod manipulations occur independently. The final total body length and mass of the larvae under high food-level were significantly higher than those in low food-level. Also larvae under high food-level grew significantly faster both in length and mass and metamorphosed faster compared to larvae under low food-level. Photoperiod had no effect on the life history traits except on the final total body length. These results indicate that amphibians are not sensible to photoperiodic cues but their critical larval life history traits are strongly influenced by food resource limitation.

The herpetofauna of Žijovo Mountain (Montenegro)

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There are no detailed studies on the herpetofauna of eastern Montenegro, and so that part of the country can be considered as poorly explored. Žijovo Mountain belongs to the Prokletije Mountain chain. They are situated in the eastern Montenegro, close to the border with Albania.

The present note provides information of amphibian and reptile species of Žijovo Mountain, and as such it contributes to the general knowledge of the Montenegrin herpetofauna. The data were collected during field surveys in 2015 and 2016. The specimens were mostly directly observed, but some of them were captured and released in the study area after determination. Determination of amphibian and reptile species was conducted using appropriate literature (Radovanović, 1951; Arnold and Ovenden, 2002; Kwet, 2009; Speybroeck J, et al. 2016).

In the study area, we recorded 10 species of amphibians (*Salamandra salamandra*, *Lissotriton vulgaris*, *Ichthyosaura alpestris*, *Bombina variegata*, *Bufo bufo*, *Bufo viridis*, *Hyla arborea*, *Pelophylax ridibundus*, *Rana dalmatina*, *Rana temporaria*) and 16 species of reptiles (*Testudo hermanni*, *Lacerta viridis*, *Lacerta trilineata*, *Podarcis muralis*, *Algyroides nigropunctatus*, *Anguis fragilis*, *Coronella austriaca*, *Natrix natrix*, *Hierophis gemonensis*, *Zamenis longissimus*, *Elaphe quatuorlineata*, *Platyceps najadum*, *Vipera berus*, *Vipera ammodytes*, *Vipera ursinii*).

As it now stands, from 17 amphibian species and 36 reptile species known for the territory of Montenegro (Džukić, 1991; Crnobrnja-Isailović and Džukić, 1995; Džukić, 1995; Tomović et al., 2004; Ljubisavljević et al., 2007; Polović and Ljubisavljević, 2010), 10 amphibian species (59%) and 16 reptile species (44%) were recorded in this area.

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Topographic factors promoting the distribution of the endemic lizard *Podarcis cretensis* in Samaria National park

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Digital Elevation Models (DSM) are widely used in landscape and evolutionary ecology. Usually, elevation and first order parameters have been calculated in order to be used as proxies for factors like solar radiation, soil water content, wind flow, climate variables and evapotranspiration over mountainous areas. Advanced 2nd order parameters or secondary topographic attributes like the Sky View Factor (SVF), Topographic Position Index (TPI), Topographic Ruggedness Index (TRI), Wind Exposition and Solar Radiation are rarely used in such studies whilst more rare appear in studies related to the species distributions in local scale.

In the present study use a DSM, coming from the Greek National Cadaster and Mapping Agency, at the spatial resolution of 5 meters in order calculate advanced topographic variables as well as in situ observations for the period 2012-2015. A Maximum Entropy (MaxEnt) model has been prepared (10 fold bootstrap); it calculated the maximum entropy of the species and provide insights in the factors that promote its distribution. A set of 9 topographic variables (Slope, Wind Exposure, TRI, TWI, SVF, Aspect, TPI, Diffuse Insolation and Direct Insolation) have been calculated using SAGA GIS and used along with 153 presence points across the Samaria National Park.

Model performance based on AUC was very good (0.911); Slope, Wind Exposure and TRI where the most important parameters according to the jackknife test along with SVF and Direct Insolation according to relative contributions of the environmental variables. The results are important for targeted conservation management of the endemic lizard.

Vital Fluorescent Staining for Neuromast Studies in Urodele Amphibians

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Neuromasts are mechanosensory organs of aquatic vertebrates, including amphibians, that function to detect vibrations in water. They consist of hair cells and surrounding support cells and are covered with a gelatinous cupula (Atema et al. 1988). Neuromasts are arranged into specific patterns to form the lateral line system.

We used a non-destructive method of fluorescent staining to analyze the distributional pattern and variation of neuromasts in the lateral line system of captive-born larvae of Italian crested newt (*Triturus cristatus*). The larvae were stained with the hair-cell-specific fluorescent dye 2-Di-4-ASP, anaesthetized in 0.3% tricaine methane-sulphonate solution with 0.2% sodium bicarbonate (pH = 7) and mounted in 0.5% agar. This cationic styryl pyridinium dye has been used for studies of neuromasts in zebrafish (Colazzo et al. 1994), but has not previously been used on urodeles.

All newt larvae survived the treatment and DiASP gave excellent results. The cranial neuromasts are grouped together in specific clusters around the eyes, nostrils, mouth, and along the hyoid apparatus, while the trunk neuromasts occur individually along three distinct lines called the ventral, median, and dorsal line. The median line continues into the tail and is present up to the tip of the tail.

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Hybridization between Danube crested newt (*Triturus dobrogicus*) and Italian crested newt (*Triturus carnifex*) in NE Slovenia

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Crested newts (*Triturus cristatus* superspecies) are a group of closely related newts with parapatric distributions, that often coexist in hybrid zones. In Slovenia two crested newt species are present: the widely distributed Italian crested newt (*Triturus carnifex*), and the Danube crested newt (*T. dobrogicus*) present in north-eastern (NE) Slovenia along Mura River floodplains.

Here we present the results of joint morphological and molecular analyses of crested newt populations from NE Slovenia. Morphological analysis using Wolterstorff index (WI) were performed in 108 specimens from 9 populations, whereas partial mitochondrial gene cytochrome *b* and 7 microsatellite loci were screened in 64 specimens. Morphological results revealed 5 pure *T. dobrogicus* populations and 4 hybrid *T. dobrogicus* x *carnifex* populations. We detected 8 different haplotypes of cytochrome *b* pertaining to *T. carnifex* and 1 pertaining to *T. dobrogicus*. mtDNA was somewhat incongruent with morphology, as we found haplotypes of both species in 3 pure *T. dobrogicus* populations according to WI and *T. carnifex* haplotypes in 3 hybrid populations according to WI. *T. dobrogicus* mtDNA was more commonly found in locations on the left river bank (36 specimens from 8 location), than on the right river bank (two specimens from 2 location). Microsatellite analysis revealed probable hybridization between both species in NE Slovenia. These results provide important data for further preparation of conservation plans for both species in Slovenia.

Phylogenetic history of *Zootoca vivipara* and the evolution of viviparity

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Common lizards (*Zootoca vivipara*) are distributed across Eurasia and the most northerly occurring reptile species. A key adaptation to their cold-adapted lifestyle is the evolution of live-bearing (viviparity). While all Northern European lineages are live-bearing, two lineages occurring in Southern Europe are egg-laying (oviparous). Phylogenetic reconstructions of different traits such as chromosomal data and mitochondrial DNA suggested that live-bearing evolved multiple times independently in *Z. vivipara*, or alternatively that oviparity re-evolved in one lineage (Odierna et al. 2004; Surget-Groba et al. 2006). However, previous phylogenetic reconstructions had only moderate support, particularly in basal nodes most crucial to understand the evolutionary history of viviparity (Surget-Groba et al. 2001; 2006).

Here, we re-construct the evolutionary history of live-bearing and egg-laying common lizards using more than 100,000 SNP markers sampling all currently known main evolutionary lineages. This includes a recently discovered Carpathian lineage that has been suggested to be related to the most basal oviparous lineage (Vekelei et al. 2015). Our phylogeny resolves some basal nodes with high bootstrap support that were previously weakly supported and change some of the previously suggested relationships. The Carpathian lineage clusters with other viviparous lineages. In summary, the genome-wide data suggests that oviparity re-evolved in the Western European oviparous lineage. This is in agreement with a previous phylogeny based on mitochondrial DNA.

Our study shows that large-scale genomic data can help to resolve some complicated phylogenetic problems, while in some cases (e.g. rapid diversification) even with larger amounts of data, phylogenomics reconstructions remain difficult to resolve.

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Natural selection and stochastic forces shape MHC class I polymorphism of lacertid lizard species

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The Major Histocompatibility Complex (MHC) has long been a model for how adaptive genetic diversity is maintained in wild populations (Sommer 2005). While pathogen-mediated selection is known to shape MHC diversity (Klein 1987), how stochasticity interacts with selection and shapes this diversity and how biogeography influences remains elusive.

Here, we used two closely related green lizard species (*Lacerta trilineata* and *L. viridis*), whose distributions span mainland and insular Greece, to address the evolutionary forces acting on the MHC in populations with different biogeographic settings. We characterized MHC class I exon 2 and exon 3 variation and neutral diversity and used this data to address the relative importance of balancing selection, convergent evolution, introgression and biogeography in shaping MHC diversity.

As expected, balancing selection was the predominant force shaping MHC diversity for both species. Interestingly, introgression from *L. viridis* contributed significantly to the MHC diversity for sympatric populations of *L. trilineata* (c.f. Nadachowska-Brzyska et al. 2012). We also found an effect of biogeographic setting on MHC polymorphism, with lower allele diversity in island populations, but no functional loss, which suggest that balancing selection has counteracted the effects of drift (c.f. Alcaide 2010). Together, our data support a strong

role for balancing selection on the MHC of these two green lizard species, with maintenance of function in spite of overall loss of variation, and introgression of diverged variants in sympatry.

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Molecular basis of alkaloid metabolism in *Dendrobates tinctorius*

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Dendrobatids sequester their poison (alkaloids) from the diet and are easy to rear in terraria, which makes them a perfect model for studying the alkaloid metabolism in amphibians.

To identify candidate genes involved in this metabolism, we performed feeding experiments coupled to transcriptomics. In our first trial, eight captive bred full siblings of *Dendrobates tinctorius* were placed in three different treatments for eight days: diet alkaloid-free (control; n = 3), diet alkaloid-free, adding alkaloids only to the last meal (single-dose; n = 2), and diet with alkaloids (multi-dose; n = 3). For treatments that involved alkaloid feeding, one of the individuals was offered an alkaloid mixture, and the others only epibatidine, an alkaloid naturally occurring in poison frogs. Samples of skin were taken for RNA-Sequencing. A transcriptome was assembled de novo and contained ~130 000 transcripts. Of these, ~35% could be annotated.

After gene expression analysis, 513 and 210 transcripts were differentially expressed in the single-dose and multi-dose treatments, respectively. In the single-dose treatment, genes related to muscular contractions, a typical effect of alkaloid poisoning, were up-regulated; however, in the multi-dose treatment, genes related to cell proliferation were up-regulated instead. In both treatments, Cytochrome P450 2A5 was up-regulated. This enzyme is known to metabolize nicotine, a compound structurally similar to epibatidine. However, we confirmed that epibatidine was sequestered and stored in the skin of *D. tinctorius* by GC/MS. The oxidation of alkaloids as an immune response might in part explain the interspecies differences in alkaloid profiles found in nature.

Morphological variation and phylogenetic relationships within the colubrid snake *Zamenis hohenackeri* (STRAUCH, 1873) provide indications for a new taxon

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Based on morphological characteristics, two subspecies of the Transcaucasian rat snake (*Zamenis hohenackeri*) are currently recognized, namely *Z. h. tauricus* and *Z. h. hohenackeri*. Both subspecies are repeatedly considered to be conspecific colour morphs and often even confused with *Z. situla*. Yet, the Transcaucasian rat snake has so far not been reviewed taxonomically.

We assessed the intraspecific morphological variation and phylogenetic relationships among specimens from different locations across its distribution range. Molecular (1191 bp mtDNA, 566 bp nuDNA) and morphological data provided sufficient evidence to support three distinct lineages within the *Z. hohenackeri* complex. These correspond to the subspecies *Z. h. hohenackeri*, *Z. h. tauricus*, and a lineage from southwest Turkey which may represent a new taxon.

Hidden treasures - recovering data on tadpole diversity from museum collections for modern scientific research on landscape and evolutionary traits

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Tadpoles exhibit a wide diversity of morphological characters. However, amphibian research still primarily aims at adults and information on the larval stage is often scarce. In scientific (museum) collections, tadpoles are often disregarded and barely processed and thus are 'hidden treasures' to scientific research. The phylogenetic and geographic distribution of tadpole morphological diversity is still barely understood. The underlying question, what determines the tadpole's morphology – shared evolutionary history or ecological factors – remains largely unanswered.

The aim of this ongoing study is to contribute to the identification of phylogenetic and geographic patterns in tadpole morphology, using larval specimens collected in different regions of the globe and housed in museum collections in Germany and Switzerland. To achieve these aims, 'classical' museum collection work and modern morphological, molecular, statistical and GIS methodology are combined. Retrieving the available data from these collections, combined with available phylogenetic and ecological data, will contribute to the understanding of patterns in tadpole morphology.

Rising from the ashes: resurrection of the Malagasy chameleons *Furcifer monoceras* and *Furcifer voeltzkowi*, based on micro-CT analyses and external morphology

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The taxonomy of the Malagasy chameleon *Furcifer rhinoceras* (GRAY, 1843) is poorly understood. The aim of this study is to clarify the taxonomic status of *Chamaeleon voeltzkowi* Boettger, 1893 and *Chamaeleon monoceras* Boettger, 1913 both only known from very few specimens collected more than 100 years ago and currently considered as synonyms of *Furcifer rhinoceras*.

Using osteological data combined with traditional morphological measurements we resurrect both taxa from the synonymy of *F. rhinoceras* as *F. voeltzkowi* and *F. monoceras*, respectively. Compared to *F. rhinoceras*, *F. monoceras* is smaller, has a relatively shorter tail, a longer and thinner rostral appendage, a gular crest and no ventral crest, whereas *F. voeltzkowi* has a smaller rostral appendage, higher casque and the dorsal crest is continuous with the tail crest.

Osteological comparisons were made using micro-X-ray computed tomography (micro-CT). We detected key differences that would be otherwise impossible to determine. We also provide a detailed morphological and osteological description of the species and conclude that *F. monoceras* and *F. voeltzkowi* are not synonyms of *F. rhinoceras*, nor should *F. voeltzkowi* be considered the same species as *F. labordi*. We strongly recommend efforts to rediscover these two poorly known taxa in order to enable additional studies and to assess their conservation status.

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Phylogeographic structure of *Rana dalmatina* BONAPARTE 1840 (Amphibia, Anura), in the southern limit of its distribution

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In this study we aim to supplement the existing knowledge (Vences et al. 2013, Canestrelli et al. 2014) on the phylogeography of *Rana dalmatina* (Amphibia, Anura), by the addition of several new samples from central and southern Greece.

A 396bp fragment of the mitochondrial Cytb gene was amplified in 31 newly collected samples, which along with 94 homologous sequences retrieved from the GenBank, were used in order to infer the phylogenetic relationships of the studied individuals. Both Bayesian Inference (BI) and Maximum Likelihood (ML) analyses produced trees with identical and highly supported topologies, which conform to the geographic origin of the samples.

A total of 36 haplotypes were found, forming two distinct lineages (Clades A and B) with an average p-distance of 6.9%. Clade A comprised haplotypes from southern Italy (Calabria), while Clade B comprised haplotypes from across the entire European range of the species. Within Clade B, three haplotype groups displaying pairwise p-distances between 0.8-1.7% were detected: B1 (H1-haplotype group), B2 (Greek group) and B3 (Peloponnisos-Lazio group). The analysis of mismatch distributions supports a long term demographic stability for Clade A and a recent sudden expansion for Clade B.

The existence of additional and well-differentiated evolutionary lineages in central and southern Greece, implies greater isolation in multiple southern refugia than previously assumed, and conforms to the “refugia-within-refugia” hypothesis (Gómez & Lunt, 2006).

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Preliminary data on composition and expression levels of Halys pitviper (*Gloydius halys*) venom toxins revealed by venom gland transcriptome sequencing

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The venom glands of *Gloydius halys* were obtained from a single specimen, four days after venom extraction. The 100-bp paired-end Illumina reads were quality trimmed and used for the de novo transcriptome assembly with Trinity. The contigs from the transcriptome assembly were further processed with TGI clustering tools to produce non-redundant transcripts at the longest possible length. Toxin-encoding transcripts were identified by blastx searches against the UniProt animal venom proteins and toxins database. Contigs matched to non-snake taxa were manually curated to ensure correct identification.

In total, 20 toxin types from 20 protein families were identified, comprising 118 unique contigs. The most abundant toxin types were snake venom metalloproteinases (SVMP; 58 contigs) and snake venom serine proteinases (SVSP; 33). However, many of the contigs identified as SVMP or SVSP were partial in length and non-overlapping, meaning that the given contig numbers are likely to be an overestimation of the number of respective genes actually expressed in the venom gland. The other identified components were 5'-nucleotidase, C-type lectin-like proteins, crotoxin-like protein, cyclotransferase, cystatin, cysteine-rich secretory proteins (CRISP), hyaluronidases, L-amino-acid oxidase (LAAO), peroxiredoxin, phospholipase B, ryncolin, serine protease inhibitors, snake venom phospholipases A2 (PLA2), snake venom vascular endothelial growth factor, translationally-controlled tumor protein, venom factors, venom nerve growth factors, venom phosphodiesterase 1, venom phosphodiesterase 2. The aforementioned toxins were presented by one to three contigs. CRISP, LAAO, SVMP, SVSP, and PLA2 were in the top 10 toxins expressed.

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The position of *Phrynocephalus rossikowi* (Reptilia, Agamidae) according to mtDNA from old museum specimen

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Phylogenetic position of *Phrynocephalus rossikowi* was never studied on the base of DNA sequences, only morphological-based hypothesis were offered (Whiteman, 1978 — on skeleton structures; Semenov, Dunayev, 1989 — on hemipenis morphology, etc.) or allozyme analysis (Likhnova, 1992). The molecular data of *Ph. rossikowi* is so scarce due to its rareness in the museum collections and difficulties in obtaining fresh material. *Phrynocephalus rossikowi* inhabits remote territories in Amu-Darya valley in Kara-Kalpak and neighbouring regions of Turkmenistan and Uzbekistan. The only specimen of this species deposited in the ZMMU collection featured dried skin collected in 1990 (ZMMU R-12795).

Molecular analysis took place in the special clean room (Laboratory of Historical DNA of ZMMU). Specific primer pairs were designed to amplify short fragments of 3 mtDNA genes (COI, cytb, ND4). We obtained 1309 b.p. in total (640 b.p. of COI, 167 b.p. of cytb, 502 b.p. of ND4). Concatenated sequences were added the alignment of other species of the genus *Phrynocephalus* from our previous work (Solovyeva et al., 2014), and analyzed using bayesian interference. *Ph. rossikowi* belongs to the clade of turan species *Ph. ocellatus* + *Ph. raddei* with high support (BI = 0.99).

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The interplay between precision and aggression during male-male competition in the poison frog *Allobates femoralis*

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In Dendrobatid frogs territorial behaviour is a widespread demeanour (Pröhl, 2005). Territorial individuals are expected to react aggressively towards intruding rivals, but in turn should inhibit such aggression towards non-threatening individuals and females. In *Allobates femoralis*, males call from elevated structures on the forest floor to announce territory possession to male competitors and to attract females (Hödl et al. 2004). Males react aggressively towards calling intruders (Narins et al 2003) and previous observations in the field have shown that they might occasionally attack approaching females.

We thus asked, if aggression comes at costs for limited precision in male *A. femoralis*. We therefore designed an experiment where we placed a model frog in 2 m distance to a calling male on the forest floor to simulate an intruding female or non-calling male. We slightly moved the model frog to attract the focal individual's attention and simultaneously presented a standardised advertisement call playback that was spatially separated from the model frog.

We found that older individuals were significantly less likely to attack the model compared to first-year adults, and that individuals that finally attacked the model approached at high speed. These results suggest that aggression comes at cost of limited precision, but this trade-off might level off with experience.

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Monitoring networks for policy-relevant amphibian species in Flanders (Belgium)

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Within the scope of the European Habitats Directive, we designed a strategy for long-term trend monitoring of policy-relevant amphibian species in Flanders, i.e. local species listed in Annexes 2 and 4 of the Directive (*Triturus cristatus*, *Alytes obstetricans*, *Pelobates fuscus*, *Epidalea calamita*, *Hyla arborea*, *Rana arvalis* and *Pelophylax lessonae*). *Salamandra salamandra* was added because of its regional conservation importance.

All known populations of the rarer species (*Salamandra salamandra*, *Pelobates fuscus*) are monitored. Generalised Random Tesselation Stratified (GRTS, Stevens & Olsen, 2004) sampling was applied for the more widespread species in order to obtain a spatially balanced design across the Flemish region. Sampling strategy and basic field methodology were described in a number of protocol documents (De Bruyn et al., 2015a, b, c). Field sampling is coordinated by the NGO Natuurpunt. Volunteer networks were developed to conduct fieldwork. A web portal was made available for attribution of volunteers to sampling sites, as well as data entry.

In 2016, monitoring networks started for *Salamandra salamandra* (4405 observed animals over 73 count events in 36 populations) and *Hyla arborea* (1210 calling males over 70 count events, 1647 larvae over 38 netting events). In 2017, *Triturus cristatus* and *Pelobates fuscus* were added, with the remaining species networks to start over the course of the following years.

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A comprehensive toolbox for ecological study of fire salamanders *Salamandra* spp. – ManderMatcher

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We wrote a VB.NET (Visual Basic) software program using coding of pattern characteristics for CMR recognition of individual animals. Patterns codes are stored into a MS Access database. Other features include measuring tools as well as data entry facilities for a myriad of data linked to both count events as well as individual animal records. The program was tested and validated with datasets of variable size, including a set over 10,000 records collected over 10 years. Data entry requires less than two minutes per record. Along with the program, a matching online application was written in the R language, using RStudio's Shiny web application frame. The app generically provides tables, graphs and exploratory data analysis from user data entered into the program.

ManderMatcher differs from other photographic CMR tools in the combination of (1) being entirely free (<https://jeroenspeybroeck.shinyapps.io/mandermatcher/>), (2) allowing filtering of the matching result, (3) allowing entry of numerous other data types (related to both count event as well as the individual records), (4) offering high-speed processing without photo editing, (5) being connected to a growing web application producing data exploration through an online user interface without demanding user knowledge of the underlying MS Access database (holding the data) or R or HTML code of the application (processing the data).

Acoustic parameters are correlated with age in male *Leptodactylus bufonius* (Anura: Leptodactylidae)

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We studied the relationship between age and acoustic parameters of the advertisement calls in *Leptodactylus bufonius* (Anura: Leptodactylidae) males from Corrientes, northern Argentina. We used skeletochronology to estimate age in 18 adult males. The advertisement calls of each male were analysed using Raven Pro software; we measured the following call parameters: (1) duration, (2) intercall duration, (3) dominant frequency, (4) fundamental frequency, and (5) frequency modulation.

The youngest male was one, while the oldest was four years old. Mean age was 2.4 years. Advertisement calls were short (0.16 ± 0.01 s), whistle-like notes with harmonic structure, emitted at intervals of 0.93 ± 0.2 s apart. The fundamental frequency was also the dominant frequency: 1403.2 ± 64.2 Hz. The call had an upwards frequency modulation of 441.03 ± 48.8 Hz (measured in the fundamental frequency). A one-way ANOVA showed that age had a significant effect on frequency modulation ($F = 4.335$, $p = 0.023$). Frequency modulation was not influenced by air temperature, humidity, or size. Age was not correlated to size.

Our results showed that males are able to convey information about their age through advertisement calls, and open up new questions for future research: are females more attracted to older males? and if they are, then what is the reproductive advantage of choosing to mate with older males? We suggest that male age in *L. bufonius* could be linked to their experience in nest construction, one of the most important features of the reproductive behaviour in this species.

Observing the enigmatic *Barbourula busuangensis* (Bombinatoridae) on Palawan Island, Philippines

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Even though *Barbourula busuangensis* was discovered almost a century ago, there is still surprisingly little known about its life and biology. Most of the works are based on museum specimens, while observations in nature are quite rare. These facts, together with the close relation to the genus *Bombina*, led me to an effort to see *Barbourula* within an extensive herpetological survey to the Philippines.

We visited the protected watershed area in Dumarao, Palawan. During one night we found 11 individuals of *B. busuangensis* (seven juveniles/subadults and four adults) and two juveniles on the next day. This number was a bit surprising according to their rarity assumed in literature.

We also noticed a few other characters not mentioned or differing compared to the published works. Colouring of the dorsal side is usually described as almost black while the adults we observed had spotted back from brown to beige. The appearance of the juveniles was very contrasting, with yellow to black pattern. The webbing of the front limbs differed in the juveniles as well as it was reaching out to the tips of the fingers, which could be related to an interesting observation concerning their habitat use. We have found the juveniles flattened against big rocks in very rapidly flowing water whereas the adults inhabited shallow and slowly flowing part upstream the brook behind a short but steep waterfall. Our findings support the need to study the ecology of this species, even though the terrain makes it quite difficult.

Courtship march not for courting? Spatio-temporal characteristics of courtship behaviour in a poison frog, *Allobates femoralis*

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Poison frogs are known for their prolonged and complex courtship behaviour, involving tactile, acoustic and visual signals. Studies show that components of courtship vary heavily across species, but generally agree that courtship is an important precursor for mating success. The neotropical poison frog *Allobates femoralis* performs a so-called “courtship march”, in which males are guiding females to oviposition sites. Compared to other poison frog species (e.g. *Ameerega braccata*, *Ameerega trivitatta*), the courtship march in *A. femoralis* lasts much longer. Assuming that this temporal variation is related to functional differences, studying the characteristics of courtship in *A. femoralis* should provide insights into its use.

To this end, we observed 29 courtship events by 15 males in a natural *A. femoralis* population in French Guiana. Seven of these males were observed repeatedly to gain more information about within- and among-individual variation in courtship behaviour. We recorded temporal, spatial and behavioural characteristics of courtship and additionally searched for already present clutches in the males’ territories.

Courtships started in the afternoon and ended on the following morning. Frogs on average moved 18.69 m (7.67–41.25 m, $n = 15$) covering an area of 14.51 m² (1.50–115.52 m², $n = 15$). Twenty-seven out of 29 courtships ended in successful oviposition, indicating that females rarely reject males once engaged in courtship.

These results suggest that the courtship march is actually not used by males to court for females, but rather to stimulate the ovulation and decide upon a high-quality oviposition site.

Breed fast, die young - Demography of a Neotropical fossorial frog

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Information regarding age- and size-related parameters is of paramount importance both to understanding the life-history of populations, and for designing effective conservation strategies. However, such base-line data lack for many species, especially for those inhabiting tropical habitats.

Using skeletochronology, we provide the first detailed information regarding the demography of the Pacific horned frog, *Ceratophrys stolzmanni*, a vulnerable, fossorial amphibian, that lives in the Tumbesian dry-forests of Ecuador.

The species shows an intense growth rate during the first activity season, both before and after metamorphosis. Sexual maturity is achieved by both sexes before the first year of life, after which growth rates abruptly decrease. Females are on average larger, but not older, than males, mean age being two years in both sexes. The rapid sexual maturation is associated with a short life-span, the observed maximum longevity being three years in males and four years in females. The short reproductive life-span, which offers few mating opportunities, might have important implications for the conservation of this species, that can be especially at risk in the case of prolonged droughts expected to affect its habitat.

Contrasting sex ratios between coexisting alternative morphs of the smooth newt, *Lissotriton vulgaris*: an example of fitness advantage?

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Facultative paedomorphosis, an environmentally induced polymorphism, that results in the coexistence of alternative phenotypes in the same reproductive habitat (Denoël et al. 2005), is frequently observed in newts and salamanders. Three cases of facultative paedomorphosis in the smooth newt, *Lissotriton vulgaris*, have been reported from Greece; one of them in an artificial permanent pond in Ioannina (NW Greece) (Sotiropoulos et al. 2017).

During 11 sampling sessions at the specific site (November 2014-May 2017), in a total of 2048 captured smooth newts the proportion of paedomorphosis was found to vary between 86-99%. Sex ratios were found to be consistently contrasting throughout the study period, with paedomorphs exhibiting a strong female-biased ratio ($\chi^2=26.91$, $p<0.01$) and metamorphs exhibiting a male-biased sex ratio ($\chi^2=33.17$, $p<0.01$).

In order to test the hypothesis that sex-specific fitness differences could explain these sex ratios (Whiteman 1997), we used the residual index (Jakob et al. 1996), as a measure of individual fitness, to compare alternative morphs in each sex, from 151 individuals (118 paedomorphs, 33 metamorphs), collected in four seasonal samplings (Autumn 2014-Summer 2015). No statistically significant differences in residual index were found between alternative morphs in both sexes (females: $t = -0.24$, $df = 64$, $p=0.8$; males: $t=1.9$, $df=83$, $p=0.05$), which however could be attributed to the lower sample size of metamorphs.

Further research focusing on other fitness components (e.g. fertility, longevity, survival rate) is needed, in order to investigate the importance of fitness differences between morphs and their role in sustaining paedomorphosis in the study site.

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Geographic correlates of dorsal coloration patterns in Palearctic vipers

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Dorsal coloration patterns in vipers have been linked to crucial ecological functions like aposematism and crypsis, but environmental and geographic causes of these patterns have been less studied. According to the "thermal melanism hypothesis", coloration in vipers might follow a trend related to the thermal characteristics of distributional ranges.

To investigate possible links between the level of dorsal pigmentation and variables related to altitude and latitude, we studied over 250 individuals from most species of the genera *Daboia* and *Vipera*. We quantified the total number of dorsal blotches along the body; we then counted the number of pigmented scales within a delimited sample area in the midline of the body; and we calculated a weighted pigmentation index (WPI) taking into account the total number of dorsal scales.

Coloration pattern seems to exhibit a strong phylogenetic inertia, as the two genera were clearly differentiated in terms of dorsal blotches, with *Vipera* presenting a higher number than *Daboia*. On the other hand, both genera included remarkable variation in the WPI within and across species. Species coloration pattern showed no relationship with individual distributional variables. However, a two-block partial least-squares analysis suggested a strong positive association between the level of pigmentation and latitude and altitude.

Our results suggest a relationship between the level of dorsal pigmentation and distributional factors, supporting the influence of thermoregulation in shaping the coloration phenotype in this group of snakes.

Sharing food to avoid the competition between lizards living in sympatry (Reptilia: Sauria) in NW Bulgaria

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The family Lacertidae is represented by nine species in Bulgaria (Stojanov et al. 2011). Sympatric occurrence of more than five species is very rare (Tzankov 2014). The studied species were the Green lizard *Lacerta viridis* (LAURENTI, 1768), the Wall lizard *Podarcis muralis* (LAURENTI, 1768), the Balkan Wall lizard *Podarcis tauricus* (PALLAS, 1814), the Meadow lizard *Darevskia praticola* (EVERSMANN, 1834), which belong to the family of Lacertidae and the single scincid lizard occurring in Bulgaria – the Snake-eyed skink *Ablepharus kitaibelii* BIBRON & BORY DE SAINT-VINCENT, 1833. Here, I report some data about seasonal and sex differentiation in the diet composition of these five sympatric lizards with similar body size.

The study was conducted in 2013 – 2014 in the Montana district in North-Western Bulgaria near Ogosta dam (location N 43.378023, E 23.210540). Five general type of habitats were described (coast, meadow, ecotone between the meadow and the forest, broadleaved forest and conifereous forest). A total of 147 individuals were capture by hand and measured. To determinate the food preferences, a total of 126 faecal samples were analyzed, which could be individually assigned to a lizard species, sex and size (length and weight) and habitat occupied by species.

All of them shown specific, seasonal, and spatial differences in their feeding preferences to avoid the competition.

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Lesser Antillean Iguana threatened by hybridization on Statia

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The Lesser Antillean Iguana (*Iguana delicatissima*) is currently listed as Endangered according to IUCN guidelines. A main reason for this listing is the threat and extent of hybridization and subsequent introgression with the non-native Green Iguana (*Iguana iguana*) which is spreading throughout the Lesser Antilles, the home range of *I. delicatissima*. The *I. delicatissima* population on St. Eustatius was believed to be pure as recently as 2016, however the capture of an *I. iguana* and several individuals with hybrid morphological features has led to concern.

We collected morphological and genetic data from more than three hundred native *I. delicatissima*, the captured *I. iguana* and four potential hybrid individuals. Our data show that, indeed, hybridization between the native *I. delicatissima* and alien *I. iguana* occurred on St. Eustatius, with possible hybrids and other *I. iguana* still present. Mitochondrial sequence data shows the captured *I. iguana* originates from either El Salvador or Honduras, although it may have come from another Caribbean island as it is known that non-native *I. iguana* populations from different regional backgrounds occur on several islands throughout the Caribbean.

This work shows the continuous spread of *I. iguana* and its threat to *I. delicatissima* throughout the Lesser Antilles. Immediate conservation actions are needed and will be discussed to eliminate the *I. iguana*/hybrid threat from St. Eustatius.

**Skeletal osteology of the lacertid lizards *Phoenicolacerta laevis* (GRAY, 1838) and *Phoenicolacerta cyanisparsa* (SCHMIDTLER AND BISCHOFF, 1999)
(Squamata: Lacertidae)**

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The adult skeletons of lacertid lizards *Phoenicolacerta laevis* and *P. cyanisparsa* are described based on cleared-and-stained specimens. In comparison with each lacertid species, there are remarkable differences in both cranial and postcranial bones. These are the shape of some cranial bones such as the premaxilla, nasal, quadrate, squamosal, jugal and dentary and the shape of the ischium and ischio-pubis opening and the number of the last presacral vertebrae with small ribs for the postcranial skeletal elements. These detailed descriptions provide as a model to compare lacertid lizards and contribute to understand squamate osteology for further studies.

Climatic niche modeling of the Stripe-necked terrapin *Mauremys leprosa* reveals low level of ecological differentiation among Moroccan populations

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Spatial distribution of taxa can be predicted using Ecological Niche Modeling (ENM) (see Elith & Leathwick, 2009). This method based on spatially-explicit environmental information and geo-referenced data, provides an estimate of the ecological niche; the multidimensional 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 (Bour & Maran, 1999), we aimed at assessing: (i) whether they inhabit different climatic niches, (ii) whether they fulfill the required conditions to be considered different incipient evolving lineages under low genetic (Fritz et al. 2005) 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 taxon. 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.

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