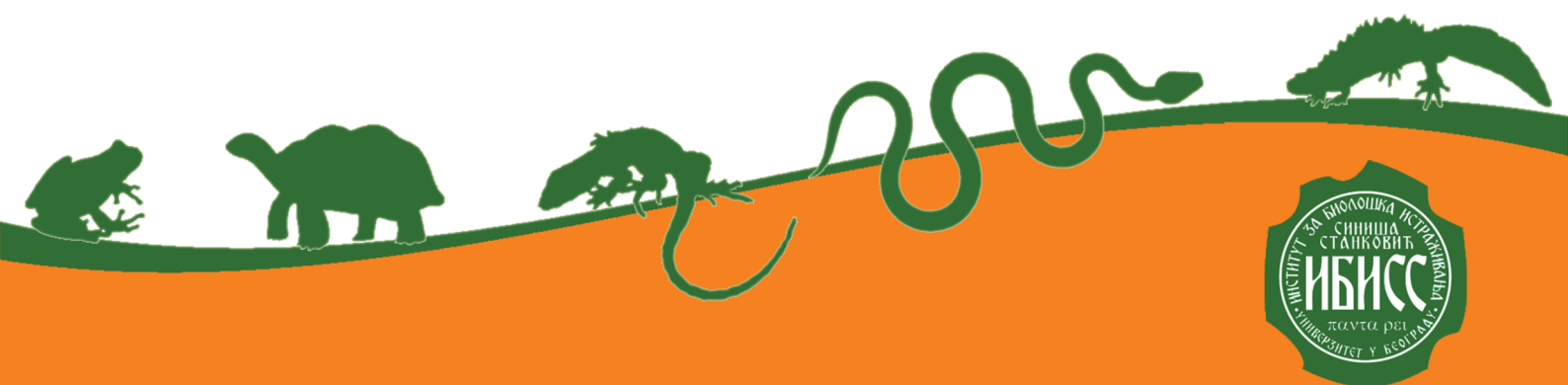




Program & Book of Abstracts

**Belgrade
2022**



Institute for Biological Research “Siniša Stanković”
National Institute of Republic of Serbia
University of Belgrade, Serbia

PROGRAM & BOOK OF ABSTRACTS

21st European Congress of Herpetology



September 5th-9th, 2022
Belgrade

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5th – 9th September 2022



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Our logo

The logo for the XXI SEH meeting was envisioned as the silhouette of a head of the most common Serbian venomous snake, the Nose-horned viper (*Vipera ammodytes*) incorporated into the traditional Pirot carpet or Pirot kilim (Pirotski ćilim) pattern. Pirot is a town in southeastern Serbia known for its rich history, geography, and biological diversity. Carpet and tapestry weaving in this region date back to the Middle Ages, and Pirot was for a long time recognized as the most important rug-making center in the Balkans. This tradition is listed as the Intangible cultural heritage of Serbia and Pirot carpet is regarded as one of our national symbols.



Welcome note

Dear Colleagues,

On behalf of the Local Organizing Committee, it is my greatest pleasure to invite you to participate at the upcoming SEH 21st European Congress of Herpetology that will take place in Belgrade, Serbia, from 5th to 9th September, 2022.

It is organized by the Societas Europaea Herpetologica and Institute for Biological Research “Siniša Stanković”, University of Belgrade – National Institute of Republic of Serbia, together with the Faculty of Biology University of Belgrade, Institute of Biology and Ecology at Faculty of Sciences and Mathematics University of Kragujevac, Department of Biology and Ecology at Faculty of Sciences and Mathematics University of Niš, Department of Biology and Ecology at Faculty of Sciences and Mathematics University of Novi Sad, Department of Biology at Faculty of Sciences and Mathematics University of Priština in Kosovska Mitrovica, Natural History Museum in Belgrade and Serbian Herpetological Society “Milutin Radovanović”.

This conference aims at bringing together scientists, nature conservation specialists and policy makers sharing a common interest in herpetology. This meeting is an excellent chance to learn about the newest research, to get into eye-to-eye contact with experts in the field and to meet old and hopefully new collaborators and friends during these challenging pandemic times. The conference will focus on various subjects like climate change and its effect on amphibians and reptiles, habitat restoration, species protection, conservation genetics, phylogeography, evolution and development, alien species and amphibian and reptile diseases.

Looking forward to your participation at 21st SEH Congress and to welcoming you at Belgrade, Serbia!

Dr. Jelka Crnobrnja-Isailović
Chair of the Local Organizing Committee



Belgrade panorama 1905. Photo by Anastas Stojanović
(Museum of Applied Art, Belgrade)

Program



Vipera ammodytes

PROGRAM OVERVIEW

Sunday, 4th September 2022			
07:45	Pre-congress excursion National Park “Djerdap”		Departure from “Ilija M. Kolarac” Endowment
Monday, 5th September 2022			
14:00 18:00	Registration		“Ilija M. Kolarac “ Main Hall
19:00	Congress Opening and Welcome Party		“Ilija M. Kolarac “ Main Hall
Tuesday, 6th September 2022			
9:30	Plenary Lecture (W. Bohme)		Room 1
10:30	COFFEE BREAK (15 min)		
	Room 1	Room 2	Room3
10:45	Biodiversity and Conservation	Anatomy and Morphology	Introduced and Invasive Species
12:30	LUNCH BREAK		
14:30	Biodiversity and Conservation (continued)	Anatomy and Morphology (continued)	Introduced and Invasive Species (continued)
15:30	Round Table: Threats on Amphibians and Reptiles in the Balkan Peninsula		Room 2
16:00	COFFEE BREAK (15 min)		
16:15	Round Table: Threats on Amphibians and Reptiles in the Balkan Peninsula (continued)		Room 2
16:15	Poster Session 1		“Ilija M. Kolarac “ Main Hall
18:15	Student Meeting		Faculty of Biology, University of Belgrade
Wednesday, 7th September 2022			
9:00	Plenary Lecture (D. Cogălniceanu)		Room 1
10:00	COFFEE BREAK (20 min)		
	Room 1	Room 2	Room 3
10:20	Biodiversity and Conservation (continued)	Reproductive systems and Life Histories	10:20 Pathogens 11:50 Ecophysiology
12:50	Congress Photo		“Ilija M. Kolarac “ Main Hall
13:00	LUNCH BREAK		
14:30	Biodiversity and Conservation (continued)	Populations in Anthropogenically Modified Environments	Ecophysiology (continued)
15:30	COFFEE BREAK (15 min)		
15:45	Ordinary General Meeting Societas Europaea Herpetologica		
Thursday, 8th September 2022			
9:00	Plenary Lecture (X. Bonnet)		Room 1
10:00	COFFEE BREAK (20 min)		
	Room 1	Room 2	Room 3
10:20	Population, Community, and Ecosystem Ecology	10:20 Ecophysiology (continued)	10:20 Biogeography and Distribution
		10:55 European Amphibian Red List Assessment Team Meeting	11:50 Population, Community, and Ecosystem Ecology 2
12:35	LUNCH BREAK		
14:30	Population, Community, and Ecosystem Ecology (continued)	Round Table: Amphibian Fungal Pathogens	European Viper Specialist Group Red List Committee Meeting
16:00	COFFEE BREAK (15 min)		
16:15	Poster Session 2		“Ilija M. Kolarac“ Main Hall
20:00	Farewell Dinner		Botanical Garden “Jevremovac“
Friday, 9th September 2022			
9:00	Plenary Lecture (L. Luiselli)		Room 1
10:00	COFFEE BREAK (20 min)		
	Room 1	Room 2	Room 3
10:20	Biology and Evolution of Behavior	Conservation and Population Genetics	Conservation and Population Genetics 2
12:35	LUNCH BREAK		
14:30	Biology and Evolution of Behavior (continued)		
16:00	COFFEE BREAK (15 min)		
16:15	Awards and Closing Ceremony		Room 1
Saturday, 10th September 2022 – Monday 12th September 2022			
7:45	Post-congress field trip: Western Serbia: Uvac Gorge, Tara National Park, Rzav River Canyon		Departure from “Ilija M. Kolarac” Endowment

Tuesday, 6th September 2022

9:30
10:30

PLENARY LECTURE (Room 1)		
Böhme, W.		
History of Herpetological Research on the Balkan Peninsula		
Chairman: Jelka Crnobrnja-Isailović		

COFFEE BREAK (15 min)

10:45
11:00

Room 1	Room 2	Room 3
Biodiversity and Conservation Chairmen: Cristophe Dufresnes, Sylvain Ursenbacher	Anatomy and Morphology Chairmen: Aaron Bauer, Aleksandar Urošević	Introduced and Invasive Species Chairmen: Raoul Manenti, Katarina Ljubisavljević
Tomović et al. Presenter: Tomović, Lj.	Cvijanović et al. Presenter: Cvijanović, M.	Pille et al. Presenter: Pille, F.
Establishment of ecological networks in Serbia – field research of batracho- and herpetofauna	What is hiding in the <i>Bufo</i> skin? Revealing of the structures in the skin of European toads using standard histological and micro-CT techniques	Predation patterns of invasive water frogs (<i>Pelophylax ridibundus</i>) in pond environments
Krása et al. Presenter: Krása, A.	Vučić et al. Presenter: Vučić, T.	Vucić et al. Presenter: Vucić, M.
Czech herpetofauna monitoring and mapping	Insights from the inside: 3D embryonic development of <i>Triturus</i> newts	Insight into the diversity of water frogs (genus <i>Pelophylax</i>) in Croatia and the cryptic invasion of Balkan water frog <i>Pelophylax kurtmuelleri</i>
Osojnik et al. Presenter: Osojnik, N.	Rupik et al. Presenter: Rupik, W.	Kruger et al. Presenter: Kruger, N.
LIFE Integrated Project for Enhanced Management of Natura 2000 in Slovenia – An expert basis for Amphibian Conservation in two selected Natura 2000 sites	Comparative 3D studies of pancreas morphogenesis in lizards of two clades: <i>Lacerta agilis</i> (Lacertoidea) and <i>Lepidodactylus lugubris</i> (Gekkota)	Is there no place like home? Response of African Clawed frog tadpoles to novel environments
Sahlean et al. Presenter: Sahlean, T.	Kaczmarek et al. Presenter: Kaczmarek, P.	Czurda et al. Presenter: Czurda, J.
The right of passage: Nature corridors for the amphibians and reptiles in Romania	Diversity of the vomeronasal sensory epithelium in non-ophidian squamates - a structural and ultrastructural study	Hybridization in crested newts (<i>Triturus</i>) at the outskirts of Vienna: limited introgression across a disrupted contact zone and sporadic introductions of non-native newts
Sacdanaku et al. Presenter: Sacdanaku, E.	Riedel et al. Presenter: Riedel, J.	Martins et al. Presenter: Rato, C.
Updating knowledge on the herpetofauna of an endangered ecosystem: the Vjosa river (Albania)	Ecomorphology of the locomotor apparatus of the genus <i>Cyrtodactylus</i> (Gekkota, Squamata)	Exotic supper: Exploring the diet composition of <i>Tarentola mauritanica</i> gecko in Madeira Island under a metabarcoding approach
Iković et al. Presenter: Iković, V.	Mármol-Guijarro et al. Presenter: Mármol-Guijarro, A. M.	Damas-Moreira et al. Presenter: Damas-Moreira, I.
The impact of traffic on the reptiles and amphibians in the Nature Park „River Zeta"	A Virtual Museum: 3D reconstruction of Ecuadorian type specimens of herpetofauna	How to outcompete a native lizard? Violence is not always the answer
Petrovan and Sutherland, Presenter: Petrovan, S. O.	Tisza et al. Presenter: Móré, A.	Oskyrko et al. Presenter: Oskyrko, O.
An evidence-based approach to conserving amphibians and reptiles in Europe	A novel geometric morphometric approach to identify snake prey vertebrae from raptor bird nests	Where are you coming from? New data on introduced reptiles in Ukraine

12:15
12:30

LUNCH BREAK

	Room 1	Room 2	Room 3
	Biodiversity and Conservation (Continued) Chairman: Judit Vörös	Anatomy and Morphology (Continued) Chairman: Milena Cvijanović	Introduced and Invasive Species (Continued) Chairman: Catarina Rato
	Polynova et al. Presenter: Polynova, G. A.	Barzaghi et al. Presenter: Barzaghi, B.	Mondino et al. Presenter: Ursenbacher, S.
14:30 14:45	Influence of overgrowing processes in sandy deserts and semi-deserts on the abundance and species diversity of reptiles	Preliminary study on differences characterizes the populations of olm (<i>Proteus anguinus</i>) in cave and those found in epigeal environment	Impact of invading species on biodiversity: diet study of the Green Whip Snake's (<i>Hierophis viridiflavus</i> , L. 1789) in Switzerland
14:45 15:00	Ballouard et al. Presenter: Ballouard, J.M.	Urošević et al. Presenter: Urošević, A.	Lužnik et al. Presenter: Lužnik, M.
	Experimental reinforcement of Hermann tortoise populations impacted by fire	Vertebral regionalization vs. morphological integration in <i>Lissotriton</i> newts	Yellow-bellied toads in garden ponds: a successful habitat creation, and a support for long-term population persistence
15:00 15:15	Mali et al. Presenter: Mali, I.	Veerappan et al. Presenter: Veerappan, D.	Ginal et al. Presenter: Ginal, P.
	Application of occupancy modeling framework to inform conservation of a small terrestrial turtle	Diet and habit explain head-shape convergences in natricine snakes	More time for aliens? Performance shifts lead to increased activity time budgets propelling invasion success
15:15 15:30	Mićanović et al. Presenter: Mićanović, A.		
	Distribution and population size of the freshwater turtles (<i>Emys orbicularis</i> and <i>Mauremys rivulata</i>) on the Montenegrin coast		
15:30 16:00	Round Table: Threats on Amphibians and Reptiles in the Balkan Peninsula (Room 2)		Chairman: Dušan Jelić
	COFFEE BREAK (15 min)		
16:15 17:00	Round Table: Threats on Amphibians and Reptiles in the Balkan Peninsula (Continued) (Room 2)		Chairman: Dušan Jelić
16:15 17:45	Poster Session 1 Sessions: Biodiversity Conservation; Introduced and Invasive Species; Pathogenes; Populations in anthropogenically modified environments; Conservation and Population Genetics; Biogeography and Distribution		"Ilija M. Kolarac" Main Hall

Wednesday, 7th September 2022

9:00
10:00

PLENARY LECTURE (Room 1) Cogălniceanu, D. Skeletochronology - a valuable tool in ectotherm population biology studies Chairman: Ana Ivanović		
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COFFEE BREAK(20 min)

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12:05
12:20

Room 1	Room 2	Room 3
Biodiversity and Conservation (Continued) Chairmen: Wolfgang Böhme, Martina Lužnik	Reproductive Systems and Life Histories Chairmen: Dan Cogălniceanu, Tijana Vučić	Pathogens Chairman: Silviu Petrovan, Andreas Maletzky
van Doorn et al. Presenter: van Doorn, L.	Dittrich et al. Presenter: Dittrich, C	Kásler et al. Presenter: Kásler, A.
At the forefront of conservation: integrating science and amphibian reintroductions in Flanders, Belgium	Parental care and paradox decisions: does relatedness play a role in nursery choice?	Optimization of heat-treatment against chytridiomycosis in <i>Bufo bufo</i> tadpoles
Denoël Presenter: Denoël, M.	Lunghi Presenter: Lunghi, E.	Ljubisavljević et al. Presenter: Vukov, T.
Facultative paedomorphic newts in the Balkans: hotspot, habitats, declines, and threats	Preliminary data on the seasonal growth rate of the Italian cave salamander <i>Speleomantes italicus</i>	Occurrence of chytrid fungus (<i>Batrachochytrium dendrobatidis</i>) and body condition in syntopic water frogs <i>Pelophylax shqipericus</i> and <i>P. ridibundus</i>
Petrovan and Sutherland Presenter: Petrovan, S. O.	Ruthsatz et al. Presenter: Ruthsatz, K.	Kok et al. Presenter: Kok, P. J.R.
Toads in trees and spots on frog. Using citizen science tools to learn new things about well-known amphibians	Microplastics have sublethal effects on amphibian larvae and lead to post-metamorphic carry-over effects: A study with polyethylene microplastics and <i>Xenopus laevis</i>	Tourist incursions predict chytrid load in amphibians from the pristine "Lost World"
Herder and Janse Presenter: Herder, J. E.	Hettzey et al. Presenter: Hettzey, A.	Leeming et al. Presenter: Leeming, S.
Successful translocation of moor frog (<i>Rana arvalis</i>) and pool frog (<i>Pelophylax lessonae</i>) in the Netherlands	Ecotoxicology in a complex world: combined effects of pesticides and pathogens during early life in an anuran amphibian, <i>Rana dalmatina</i>	Ectoparasitism in Polystomatidae (Neodermata, Monogenea): phylogenetic position and mitogenome of <i>Sphyrnura euryceae</i> , a parasite of the Oklahoma salamander
Janse et al. Presenter: Janse, J.	Muraro et al. Presenter: Muraro, M.	
Conservation practices of yellow-bellied toad and midwife toad in The Netherlands	Drivers of sexual dimorphism variation across populations of the Italian wall lizard at different spatial scales	Ecophysiology Chairman: Nataša Tomašević Kolarov
Brito et al. Presenter: Brito, J. C.	Roitberg et al. Presenter: Roitberg, E. S.	Stückler et al. Presenter: Stückler, S.
Diversity, distribution and conservation of the West African Crocodile in Mauritania	Variation in body size and sexual size dimorphism in the lizard <i>Zootoca vivipara</i> : The effects of reproductive mode revisited	Evidence that catecholaminergic systems are evolutionarily co-opted to mediate dynamic colour change during explosive breeding events in toads
Billy et al. Presenter: Billy, G.	Bouazza et al. Presenter: Bouazza, A.	Kijanović et al. Presenter: Kijanović, A.
Strict nature reserve status and snake population maintenance	Elevation influences reproductive traits and maternal conditions in an alpine gecko	Desiccation stress response of <i>Bombina variegata</i> tadpoles
Zotos et al. Presenter: Zotos, S.	Zeigler et al. Presenter: Ziegler, C.	Carretero et al. Presenter: Carretero, M. A.
Predicting mortality risks of colubrid snakes on Cyprus roads using habitat suitability modelling	Optimal body mass-length ratio during hibernation for <i>Emys orbicularis</i> (Linnaeus, 1758) – European Pond Turtle	Has fundamental niche shifted in the Aeolian <i>Podarcis</i> ? An ecophysiological investigation

	Room 1	Room 2	Room 3
12:20	Stark and Janssen Presenter: Stark, T.		Čubrić and Crnobrnja-Isailović Presenter: Čubrić, T.
12:35	Integrating citizen science with grass snake conservation: six-year results of an online management tool for grass snake breeding heaps in the Netherlands		Limited body temperature variation in the <i>Vipera ammodytes</i> (Linnaeus, 1758) during six years study
12:50	Congress Photo “Ilija M. Kolarac” Main Hall		
13:00			

LUNCH BREAK

	Biodiversity and Conservation (Continued) Chairman: Dušan Jelić	Populations in Anthropogenically Modified Environments Chairman: Dragana Cvetković	Ecophysiology (Continued) Chairman: Savvas Zotos
14:30	Mebert et al. Presenter: Mebert, K.	Vacher et al. Presenter: Vacher, J. P.	
14:45	Dwarf vipers come out big: perspectives on distribution, taxonomy, and conservation of grassland vipers in West Asia	Field hedgerows and wood edges are critical habitats for amphibians and reptiles in agricultural landscapes: a case study in western France	
14:45	Martínez-Freiria et al. Presenter: Martínez-Freiria, F.	Clement et al. Presenter: Clement, V.	Jovanović Glavaš et al. Presenter: Jovanović Glavaš, O.
15:00	IUCN Red List assessment of the Western Mediterranean vipers, <i>Vipera latastei</i> and <i>Vipera monticola</i> . New perspectives derived from a taxonomic update	Site occupancy monitoring and influence of landscape features on the occurrence of the European treefrog (<i>Hyla arborea</i>) in the Upper Rhine valley	Effects of artificial light at night on green toad development
15:00	Rák et al. Presenter: Rák, G.	Burgstaller et al. Presenter: Burgstaller, S.	Žagar et al. Presenter: Žagar, A.
15:15	Effects of different grassland management regimes on the abundance of the endangered Hungarian meadow viper (<i>Vipera ursinii rakosiensis</i>)	The urban green toad: history, population structure and ecological adaptations of an inner-city population in Central Europe	Can we use metabolic parameters to understand thermal preferences? A case study of five lacertid lizards
15:15	Riaño et al. Presenter: Riaño, G.	Stamenković et al. Presenter: Crnobrnja-Isailović, J.	Iryshkov et al. Presenter: Nikolaev, O. D.
15:30	Genomics reveal mitonuclear discordance and introgression in the desert-adapted vipers of the genus <i>Cerastes</i>	Pesticides in the eggshells and nests of <i>Testudo hermanni</i> in a complex habitat system	Thermobiological basis of cooperation in small heliothermic lizards of the genus <i>Darevskia</i>
	COFFEE BREAK(15 min)		
15:45	Ordinary General Meeting Societas Europaea Herpetologica		
18:00			

Thursday, 8th September 2022

9:00
10:00

PLENARY LECTURE (Room 1)		
Bonnet, X.		
A whim of the gods: reptiles in the Garden of Eden		
Chairman: Ljiljana Tomović		

COFFEE BREAK (20 min)

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Room 1	Room 2	Room 3
Population, Community and Ecosystem Ecology Chairmen: Luca Luiselli, Stefan Zamfirescu	Ecophysiology Chairman: Anamarija Žagar	Biogeography and Distribution Chairman: Jose Brito
Arntzen Presenter: Arntzen, J. W.	Damm et al. Presenter: Damm, M.	Bauer and Stanley Presenter: Bauer, A.
Atlas data reveal pattern and process of species replacement	Diverse like a Cocktail Bar – Snake Venom Proteomics of the Old World Vipers	Mapping the Amphibians and Reptiles of Namibia
Manenti et al. Presenter: Manenti, R.	Lakušić et al. Presenter: Lakušić, M.	Bouazza et al. Presenter: Bouazza, A.
Activity of the olm (<i>Proteus anguinus</i>) in surface habitats: ecological and evolutionary insights	Food for thought: Venom composition of <i>Vipera ammodytes</i> from the island and mainland population is likely related with diet	Evaluating the impact of water holes during the covid-19 lockdown on Herpetofaunal community in arid regions southern Morocco
DiGregorio and Lunghi Presenter: Di Gregorio, M.	10:55 – 12:35 European Amphibian Red List Assessment Team Meeting	Baptista et al. Presenter: Baptista, N. L.
Trophic niche of an epigeal population of <i>Speleomantes sarrausensis</i>		New Bufonids from understudied Angolan centers of endemism
Lunghi et al. Presenter: Corti, C.		Talavera et al. Presenter: Talavera, A.
The diet of two sympatric salamanders, the Fire salamander and the Italian cave salamander		Genomic insights into a critically-endangered glacial relict – the Montseny brook newt
Breka et al. Presenter: Breka, K.		Maddock et al. Presenter: Maddock, S. T.
Is trophic differentiation of green frogs in <i>Pelophylax ridibundus</i> - <i>P. esculentus</i> - <i>P. lessonae</i> population systems in South Banat, Serbia related to habitat suitability?		Evolutionary patterns in New Guinea small-eyed snakes (<i>Micropechis ikaheka</i>) inferred with museomic approaches
Kirkpatrick et al. Presenter: Kirkpatrick, B.		Burriel-Carranza et al. Presenter: Burriel-Carranza, B.
Combining models and field observation to predict impacts of drought on temperate amphibians		Using genomics to reveal the diversity and biogeography of the Hajar Mountains' endemic reptiles
Blažević et al. Presenter: Blažević, M.		Population, Community, and Ecosystem Ecology 2 Chairman: Olga Jovanović Glavaš
Assessing microhabitat preferences and population density using capture-mark-recapture method for <i>Telescopus fallax</i> (Fleischman, 1831) in Central Dalmatia		Limnious et al. Presenter: Limnious, A.
		Does climate crisis threaten Greek Amphibians? A species distribution modelling and time-series analysis approach

	Room 1	Room 2	Room 3
	Mao and Lin Presenter: Mao, J. J.		Lo Parrino et al. Presenter: Lo Parrino, E.
12:05 12:20	A 12 years monitoring of <i>Trimeresurus stejnegeri</i> population during extreme climate events in northeastern Taiwan		Factors influencing occupancy and reproduction probabilities in amphibians: an application of multistate occupancy models
	Stamatiou et al. Presenter: Stamatiou, M.		Koller Šarić et al. Presenter: Koller Šarić, K.
12:20 12:35	Impact of climate change on the distribution of the island endemic <i>Natrix natrix cyprica</i>		Population size estimation of the Yellow-bellied Toad (<i>Bombina variegata</i>) at two locations along the Sutla River (Maceljaska gora and Hum na Sutli), Croatia
LUNCH BREAK			
	Population, Community and Ecosystem Ecology (Continued) Chairman: Claudia Corti	14:30 - 16:00 Round Table Amphibian Fungal Pathogens (Chairmen: Tanja Vukov, Katarina Ljubisavljević)	14:30 - 16:00 European Viper Specialist Group Red List Committee
	Szabolcs et al. Presenter: Szabolcs, M.		
14:30 14:45	The effects of topography and climate fluctuations on the species and lineage richness of amphibians and reptiles living in the Balkan Peninsula		
	Dajčman et al. Presenter: Dajčman, U.		
14:45 15:00	Lacertid competition: Insights from mechanistic modelling		
	Schmitz et al. Presenter: Rodder, D.		
15:00 15:15	Spatiotemporal patterns of habitat use by the sand lizard (<i>Lacerta agilis</i> Linnaeus, 1758): Effects of climatic seasonality?		
	Rocha and Maia-Carneiro Presenter: Rocha, C. F. D.		
15:15 15:30	Diverging niche dimensions in two syntopic lizard genus <i>Tropidurus</i> living in an area of the Caatinga Biome in Northeastern Brazil		
	Arsovski et al. Presenter: Arsovski, D.		
15:30 15:45	Brutal tortoise kismet: causes and consequences of extreme sex-ratio bias and insufferable sexual conflict		
COFFEE BREAK (15 min)			
16:15 17:45	Poster Session 2 Sessions: Population, Community, and Ecosystem Ecology; Ecophysiology; Biology and Evolution of Behavior; Paleoherpetology; Reproductive Systems and Life Histories; Anatomy and Morphology		“Ilija M. Kolarac” Main Hall
20:00 23:00	Farewell Dinner Botanical Garden “Jevremovac”		

Friday, 9th September 2022

9:00 10:00		PLENARY LECTURE (Room 1) Luiselli, L. Community structure and conservation ecology of West African turtles and tortoises: a synthesis of 25 years of data Chairman: Tanja Vukov		
		COFFEE BREAK (20 min)		
		Room 1	Room 2	Room 3
		Biology and Evolution of Behavior Chairmen: Xavier Bonnet, Miguel Carretero	Conservation and Population Genetics Chairmen: Ulrich Joger, Fernando Martinez-Freiria	Conservation and Population Genetics 2 Chairman: Daniel Jablonski, Jan Willem Arntzen
10:20 10:35		Glogoški et al. Presenter: Glogoški, M.	O'Brien et al. Presenter: O'Brien, D.	Dufresnes Presenter: Dufresnes, C.
		Sociability and aggressivity of insular Italian wall lizard vary in dependence on ecological conditions	From trees to <i>Triturus</i> – why we need to rethink our approach to protected areas for amphibians and reptiles	Genomic phylogeography as a lie detector to traditional molecular taxonomy in amphibians
10:35 10:50		Gojak et al. Presenter: Lisičić, D.	Dursun et al. Presenter: Dursun, C.	Stanković et al. Presenter: Stanković, D.
		The effect of chemosensory enrichment on habituation in captive common wall lizards (<i>Podarcis muralis</i>)	The intraspecific mtDNA differentiation of <i>Bufo verrucosissimus</i> (Pallas, 1814)	Extended insights into genetic diversity of alpine newts in the Julian Alps (Slovenia)
10:50 11:05		Galoyan et al. Presenter: Galoyan, E.	Petzold et al. Presenter: Petzold, A.	
		The consequences of interspecies relations between different species of rock lizards (Darevskia)	Conquering taxonomic impediments within cophyline frogs (Anura: Microhylidae: Cophylinae) using a type-specimen explicit protocol for integrative species delimitation	
11:05 11:20		Sopilko and Golyan Presenter: Sopilko, N. G.	Di Canio et al. Presenter: Di Canio, V.	Recknagel et al. Presenter: Recknagel, H.
		Sisterhood in reptiles: how and why females of viviparous lizard <i>Zootoca vivipara</i> aggregate together?	Reliability of de novo assembly of ddRAD sequences for population genetics in non-model species	Multiple independent transitions to cave life and high diversity in the olm
11:20 11:35		Tan et al. Presenter: Tan, W. C.	Antoniou et al. Presenter: Antoniou, T.	Ambu et al. Presenter: Ambu, J.
		Bite or run? Ecomorphological relationship in African agamas	Patterns of Genetic Diversity in Two Endemic Lizard Populations of Cyprus	The evolution of midwife toads (<i>Alytes</i>) revisited: a genomic approach
11:35 11:50		Golubović et al. Presenter: Golubović, A.		Velenská and Šmíd Presenter: Velenská, D.
		Turtles racing for resources: Locomotor performances of sympatric populations of <i>Emys orbicularis</i> and <i>Mauremys rivulata</i>		Systematics and biogeography of the colubrid genus <i>Platyceps</i>
11:50 12:05		Maričić et al. Presenter: Maričić, M.	Meier et al. Presenter: Meier, N.	
		What steers you to turn over? Analyses of European Pond Turtle righting success and performance	Lack of reproductive isolation between the two subspecies of the European whip snake <i>Hierophis viridiflavus</i>	

	Room 1	Room 2	Room 3
	Bjelica et al. Presenter: Bjelica, V.	Joger and Hugemann Presenter: Joger, U.	Spaseni et al. Presenter: Spaseni, P.
12:05 12:20	Drop dead gorgeous: death feigning behaviour in three distinct colour morphs of dice snakes	The Alpine clade of <i>Vipera berus</i> – a new but still puzzling taxon for the European snake fauna	So close, no matter how far': A remarkable new record extends the distribution, but not the genetic diversity of the Moldavian meadow viper (<i>Vipera ursinii moldavica</i>)
	Halpern et al. Presenter: Halpern, B.		Mizsei et al. Presenter: Mizsei, E.
12:20 12:35	Testing the effects of captive environment on individual variation in feeding behaviour of juvenile Hungarian meadow vipers (<i>Vipera ursinii rakosiensis</i>)		Conservation genomics shows limited negative effects of isolation in an endangered venomous snake living on sky islands

LUNCH BREAK

	Biology and Evolution of Behavior (Continued) Chairman: Ana Golubović
	Strugariu and Martin Presenter: Strugariu, A.
14:30 14:45	Factor in fear? Disentangling the roles of exploitative versus interference competition in disruptive selection
	Zotos Presenter: Zotos, S.
14:45 15:00	Decrypting reptiles' behaviour through small size accelerometers
	Sos et al. Presenter: Sos, T.
15:00 15:15	Consistency on the cold-hot types continuum: thermoregulation and personality study in viviparous lizard
	Petrović et al. Presenter: Petrović, S.
15:15 15:30	Every snake for itself: Antipredator behavior of two syntopic populations of <i>Natrix</i> snakes
	Mirč et al. Presenter: Mirč, M.
15:30 15:45	How good are European tree frog (<i>Hyla arborea</i>) tadpoles in hiding?

COFFEE BREAK (15 min)

16:15 17:45	Awards and Closing Ceremony (Room 1)
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Plenary speakers



Vipera ursinii



Prof. Wolfgang Böhme

University of Bonn, Germany

Born November 1944 near Kiel, Schleswig-Holstein (Germany), I visited basic school and high school in Kiel. From 1965 I studied zoology, botany and paleontology at the University of Kiel and reached my doctoral degree in 1971 with a dissertation on genital morphology in lacertid lizards. A few weeks after I got the position of a herpetological curator at the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK) in Bonn which I held until my retirement in 2014. In these years I increased the collection from less than 10.000 to ca. 100.000 specimens. Subsequently additional duties were added to the curatorial function: From 1992 I became Head of the Vertebrate Department and Vice Director of the Institute and Museum.

Research in systematics, ecology and biogeography of amphibians and reptiles, with a main focus on lizards. Founder and editor of „Handbuch der Reptilien und Amphibien Europas“, having edited the first five volumes from 1981 to 1999. Research travels mainly to West and Central Africa including a trans-Sahara mission. Currently (co)author of 775 scientific publications.

Since 1980 teaching zoology at the University of Bonn, followed by the habilitation degree in 1988. Since then supervision of more than 140 master students and 46 doctoral students, plus 30 candidates for state examen. Professorship awarded in 1996. Members of my working group were active in biodiversity research of the tropics of Central and South America (Costa Rica, Venezuela, Peru, Bolivia, Chile), of Africa (Gambia, Guinea Bissau, Benin, Cameroon, Gabon, Kenya, Tanzania, Zambia) and Madagascar, and of SE Asia (Cambodia, Vietnam, Indonesia).

September 1979 host and founding member of the „Societas Europaea Herpetologica“ (SEH) which publishes „Amphibia-Reptilia“, simultaneously founded in Bonn at ZFMK. Elected for president of the society 1993 in Barcelona, re-elected for another four years in Prague. Before, from 1983 to 1991 Chairman of the „Deutsche Gesellschaft für Herpetologie und Terrarienkunde“ (DGHT) which grew from ca. 2000 members to over 5000, making it the largest association of its discipline in the world. Since 1994 honorary member of this society.

Since 2011 elected honorary foreign member of the „American Society of Ichthyologists and Herpetologists“ (ASIH) and 2021 elected honorary member of the Russian „Nikolsky Herpetological Society“. From my retirement 2014 onwards I am working as a honorary volunteer in ZFMK's Herpetology Section.



Prof. Dan Cogalniceanu

University of Constanca, Romania

Dan Cogălniceanu was born in 1960 in Bucharest, Romania. He has studied biochemistry at the University of Bucharest and obtained his PhD in Ecology in 1997 on amphibian communities from the Lower Danube floodplain. He was an assistant professor for 15 years at the University of Bucharest, Department of Ecology. Presently he is professor at the University Ovidius Constanța (Romania) and a visiting professor at Central European University (Vienna). He lectured for two semesters at Eberswalde Technical University (Germany) as a DAAD scholar, was a Senior Fulbrighter in the USA and a Prometeo investigator (Ecuador). His work is mostly focused on the conservation, ecology, biogeography and evolutionary biology of amphibians. Work experience outside Europe includes USA, Qatar and Ecuador. He has published more than 100 research papers and is author of several books and chapters. He is a member of SEH since 1991, member of the SEH Conservation Committee and SSC/IUCN and was chair of DAPTF for Romania. He was a member of the editorial board of *Amphibia-Reptilia* (1996-2000) and currently is an associate editor of *Herpetological Conservation and Biology*.



Dr. Xavier Bonnet

CNRS & University of La Rochelle, France

Xavier Bonnet, born in 1963, is a Senior Researcher employed by the CNRS, its laboratory named Centre d'Etudes Biologiques de Chizé is located in France (UMR 7372 CNRS-Université de la Rochelle). During the past 30y, XB studied snakes, tortoises and humans mostly in the field (e.g. France, Morocco, Togo, Australia, New Caledonia, North Macedonia). Its approach combines mark recapture monitoring with eco-physiological investigations. XB regularly shifts from fundamental to conservation issues. A substantial part of its activity is devoted to environmental education. Besides long term field surveys, XB is the PI of a project on the swimming ability of snakes and on a project about ophiophobia.



Dr. Luca Luiselli

Institute for Development, Ecology, Conservation and Cooperation, Roma, Italy & Rivers State University of Science and Technology, Nigeria

Luca Luiselli, born in 1965, Italian and Nigerian citizenship, is an ecologist mainly working in Africa on reptile communities, turtle and tortoise conservation, bushmeat trade and global ecology and conservation. Concerning European species, it has focused his scientific interest mainly on viper ecology and snake community ecology. He currently teaches “Tropical Ecology” at the University Roma Tre (Rome), “Population Ecology” and “Biostatistics” at the University of Lomé (Togo) and at the Rivers State University of Science and Technology (Port Harcourt, Nigeria), and is a visiting professor at the Universities of Ouagadougou, Dedoudou (Burkina Faso) and Juba (South Sudan). During his career, he has won over 30 international research grants, mainly for studies on African turtle conservation ecology. He has published so far over 300 peer-reviewed papers, many of them on reptile ecology and conservation.

Plenary lectures



Natrix tessellata

Plenary lecture

History of herpetological research on the Balkan Peninsula

Böhme W.^{1,*}

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Southern Europe consists mainly of three large and separate peninsulas shaping the northern coastline of the Mediterranean Sea. Of these, the Iberian peninsula in the west harbours two countries, viz. Portugal and Spain, while the central Apenninian peninsula is made up by just one country, viz. Italy. The Balkan peninsula, in contrast, consists of significantly more countries, including independent states established upon the breakup of former Yugoslavia, then Albania, Bulgaria and Greece. Geographically, as defined by the famous Serbian geographer Jovan Cvijić, also the south of Romania and the European part of Turkey („Turkish Thrace“) have to be added. This historically induced multinational cultural diversity had also great influence on the history of science, including herpetology.

The global birthplace and also the name-provider of the term herpetology was Greece and its language, where for instance Aristotle (384-322 BC) wrote an astonishingly exact description of the chameleon while the role of snakes was more a mythological one, nonetheless influential for the entire realm of the Greek-Roman antiquity. But since the origin of modern herpetology by the fundamental work of Carolus Linnaeus, mostly researchers from more northern parts of Europe started to study Balkan herpetology.

The lecture will review the main contributors to the herpetology of the Balkan peninsula. They will be discussed countrywise, according to the contemporary geographic delimitations. A focus is laid on the remarkable shift from initially mostly foreign researchers towards autochthonous herpetologists taking care by themselves for the exploration of their respective countries. An overview of the zoogeographical importance of Balkan amphibians and reptiles is given, followed by a summary of important endemic species living in this herpetological diversity hotspot.

Plenary lecture

Skeletochronology – a valuable tool in ectotherm population biology studies

Cogălniceanu D.^{1,*}

¹University Ovidius Constanta, Constanta, Romania

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Accurate age determination is vital for understanding life-history strategies and their evolutionary implications. Skeletochronology, alongside capture-mark-recapture (CMR) studies, is a widely used non-lethal method to estimate age and growth-related parameters in amphibians and reptiles. Skeletochronology allows to determine the age of individuals by examining their bones, based on the presence and number of annual lines of arrested growth deposited in the bone tissue during periods of inactivity like hibernation and/or aestivation. Additionally, changes in the rate of growth as a result of transitions from a life-stage to another can be detected in the bone structure, allowing for example to estimate the age when an individual becomes capable to produce offspring. Several age-related parameters can be estimated from the cross sections: average lifespan, longevity (i.e., maximum age observed), and age at sexual maturity (considered either as the youngest breeding age class or inferred from the bone growth pattern).

The method of skeletochronology became established and widespread since the '90s through the studies of Castanet and Smirina, who proposed a standardized protocol. An extensive database on the subject includes 500 papers on amphibians, 100 on turtles, and over 200 on other reptiles (snakes, lizards and crocodiles). This encouraged their use in macroecological and evolutionary studies, either focused on single wide-spread species with many populations (e.g., *Rana temporaria*, *Epidalea calamita*) or large scale studies involving tens of species (e.g., altitudinal or latitudinal variation in lifespan, range and/or body size in relation to age). Some limitations of the method were highlighted, mostly related to the difficulty of estimating the age in old individuals (>15 yrs). Nevertheless, compared to CMR, skeletochronology is a faster and more reliable method for estimating individual age in amphibians and reptiles, despite its limits and difficulties.

Plenary lecture

A whim of the gods: reptiles in the Garden of Eden

Bonnet X.^{1,*}

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Golem Grad Island (Prespa Lake, North Macedonia) exhibits a singular physiognomy. Sheer cliffs separate a plateau from a rocky shore; abundant ruins provide shelters to reptiles. This well-structured landscape creates contrasted habitats and microhabitats. Four reptile species that are common in the Prespa region are particularly abundant on Golem Grad: Hermann's tortoises, dice snakes, nosed-horned vipers and wall lizards. The tortoises and the snakes were subjected to long-term mark recaptures. Comparisons with continental populations show that the unique conditions that prevail on the island shaped the ecology, phenotype and behaviours of these reptiles in spectacular way. Gigantism, dwarfism, or profound shifts in reproductive output and of mating systems illustrate how selective forces can sculpt organisms. The large numbers of animals that can be rapidly processed open avenues to pose various questions and to set up original experiments. In combination to the beauty of Golem Grad, this likely explains why Golem Grad became an extraordinary outdoor laboratory for students and researchers passionate by wildlife and evolutionary ecology. As in almost all ecosystems, the exceptional fauna of Golem Grad is threatened by legal and illegal human activities.

Plenary lecture

Community structure and conservation ecology of West African turtles and tortoises: a synthesis of 25+ years of data

Luiselli L.^{1,2,3,*}

¹Institute for Development, Ecology, Conservation and Cooperation, Rome, Italy

²Department of Animal and Environmental Biology, Rivers State University of Science and Technology, Nigeria

³Laboratoire d'Ecologie et d'Ecotoxicologie, Faculté des Sciences, Université de Lomé, Togo

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From 1994 to 2022, I conducted and/or supervised many field projects on community ecology of freshwater turtles and tortoises in West Africa, spending at least 100 days per year in the region and including university students' projects, environmental projects by large companies, applied ecology and conservation ecology projects funded by large foundations and conservation organizations, and purely academic researches as well. These field studies were conducted in a suite of countries and habitats, including Nigeria, Benin, Togo, Ghana, Ivory Coast, Burkina Faso, Mali, Niger as well rainforests, mangroves, deciduous forests, Guinea savannahs, Sudan savannahs and the Sahel. These studies varied in terms of length and efficacy of the sampling, but provided some noteworthy patterns. First of all, we observed that the community structure of turtles and tortoise populations was much more constant in savannahs than in forest areas, including their river systems. So, while there were remarkable variation in the assemblage composition and relative abundance of the various species inside forest areas even in nearby sites, on the other hand there was a remarkable homogeneity of the same aspects in the savannahs. Our study also revealed some noteworthy conservation ecology aspects for the West African chelonians, especially for *Kinixys* spp. and *Cyclanorhis elegans*. All these aspects will be examined and highlighted in the present speech.

Session: Anatomy and morphology



Triturus macedonicus

Anatomy and morphology

Oral presentation

Preliminary study on differences characterizes the populations of olm (*Proteus anguinus*) in cave and those found in epigeal environment

Barzaghi B.^{1,2,*}, Ficetola G.F.¹, Mauri E.^{3,4}, Restaino M.³, Lombardi B.¹, Mangiacotti M.⁵, Manenti R.^{1,2}

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The olm (*Proteus anguinus*) is considered the most representative example of all the stygobiont fauna. Nevertheless, it is also reported in some spring habitats of Friuli Venezia Giulia (North-eastern Italy). Springs are intriguing habitats that border two strongly distinct environments: surface and underground. They may provide distinct environmental pressures to stygobiont animals promoting new adaptations.

The aim of this study is to verify if there are differences in the structural morphology of the head and in the presence of melanin in the skin between the olms that are found in caves compared to the individuals found in the external environment.

During 2021, 28 olms, 18 from underground habitats and 10 from external sites, were photographed in standard conditions with a metric reference. For each individual, only the cephalic region has been considered. The pictures were processed using TPSdig software. For each picture we placed 2 landmark homologues, matching the emergence of the gills branches, and 48 semi-landmark to define the contour of the head. With regard to melanin 2 mm of skin were removed from the tail of the olm and analyzed through a spectrophotometer. The results indicate a significant effect of the environment on the shape of the olm's head, in fact the shape of the head appears more elongated in the front and narrower in the parietal region in the olms coming from the hypogean environment. In addition, the olms that use the epigeal environment show higher melanin levels in their skin.

Oral presentation

What is hiding in the *Bufo* skin? Revealing of the structures in the skin of European toads using standard histological and micro-CT techniques

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In the skin of many terrestrial anuran taxa, a specific acellular mineralized tissue layer (the Eberth-Katschenko layer) has been documented. This layer is generally positioned in between the stratum spongiosum and the stratum compactum of the dermis and has a role in reducing water loss. Here, we document and compare the amount of calcium deposition in the skin of the head and the parotoids (the external skin glands) in males and females of the common toad *Bufo bufo* and the spined toad *B. spinosus*. *Bufo bufo* and *B. spinosus* are morphologically similar and both show a conspicuous sexual dimorphism. Using standard histological techniques, we detected calcium as an amorphous ‘ground substance’ located in the stratum spongiosum, just above the Eberth-Katschenko layer. We observed large variability in the number of calcium deposits between the species and the sexes. Using micro-computed tomography (micro-CT) we were able to quantify the level of the toad skin calcification. *Bufo spinosus* females stand out compared to conspecific males and *B. bufo* on account of a strong calcification of the dorsal and ventral skin and the parotoid glands. Species and sexes significantly differed in the amount of calcium deposits (Fisher’s exact test, $p < 0.001$) and pairwise comparisons showed that groups differed from one another ($p < 0.05$), suggesting size and sexual dimorphism in these traits. We conclude that micro-CT scanning is useful for the quantification of calcified structures in the anuran skin, and keeps a promise for further studies on taxonomic and geographic variation.

Oral presentation

Diversity of the vomeronasal sensory epithelium in non-ophidian squamates – a structural and ultrastructural study

Kaczmarek P.^{1,*}, Kowalska M.¹, Rupik W.¹

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The vomeronasal sensory epithelium (VSE) of adult caenophidian snakes is divided into hundreds of tall columns containing bipolar neurons and the undifferentiated basal cells. These peculiar structures are formed by undulations of the basal lamina and the intrusion of the underlying, well-vascularized, connective tissue towards the apical part of the VSE. The role of the VSE columns is not fully understood. It has been suggested that it is an adaptation for better neuron nutrition of hypertrophied ophidian VSE. The presence of the VSE columns in lizards is unclear. However, the intrusion of the connective tissue into the VSE has been noticed in some species. In this study, we analyzed the structure and ultrastructure (TEM, SEM) of the VSE of late (pre-hatch) embryonic stages representing three large non-ophidian squamate clades: the leopard gecko *Eublepharis macularius* (Gekkota), mourning gecko *Lepidodactylus lugubris* (Gekkota), brown anole *Anolis sagrei* (Iguania) and sand lizard *Lacerta agilis* (Lacertoidea). Results of this study showed that at least three states of characters, considering the relationship of the connective tissue to the VSE, may be distinguished: 1. no intrusion, 2. irregular compartments of connective tissue, and 3. short ophidian-like VSE columns. Moreover, the other features of VSE vary among the studied species: the presence of the olfactory pigment, neuron packing density, and location of undifferentiated cells.

Anatomy and morphology

Oral presentation

A Virtual Museum: 3D reconstruction of Ecuadorian type specimens of herpetofauna

Mármol-Guijarro A.C.^{1,*}, Stacey-Solis M.¹, Pazmiño-Otamendi G.I.¹, Varela-Jaramillo A.L.^{1,2}

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Natural history collections are central for the study of the extant biodiversity on planet Earth, where each voucher specimen constitutes an invaluable unique spatiotemporal record. Yet, access to these specimens is limited only to specialists in order to reduce the specimen degradation as much as possible. There is a growing interest in using 3D models of museum specimens for morphometric, taxonomic and evolutionary studies instead of the physical voucher, which are available on the web. Here, we present a case study generating three dimensional models of relevant herpetological type material deposited in the Museo de Zoología QCAZ, Ecuador, using photogrammetry. Morphometric comparisons using linear regressions between measurements taken from the 3D model and from the type specimen indicate that the measurements were highly correlated. Our digital specimen may be used instead of the type specimen for such studies, which would likely increase its useful life and will be uploaded in the Bioweb, an open access initiative of museum QCAZ. Therefore, our project contributes to the democratization of knowledge of Ecuadorian herpetological biodiversity, increasing the number of cooperative projects between institutions, allowing researchers to allocate travel expenses to other research-related activities, and enabling natural history collections to create online repositories containing digital vouchers that could serve as backups in case of lost or destruction.

Anatomy and morphology

Oral presentation

Ecomorphology of the locomotor apparatus of the genus *Cyrtodactylus* (Gekkota, Squamata)

Riedel J.^{1,2,*}, Higham T.³, Grismer L.⁴, Russell A.⁵, Reinhold K.², Rödder D.¹

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Adaptive radiations, clades of closely related species diversified along one or more morphological and/or ecological niche axes, garner considerable interest from evolutionary biologists. Lizard radiations diversifying along spatial niche space are hypothesised to exhibit distinct changes in body length and relative limb dimensions. One hypothesis predicts that cursorial species have relatively longer hind limbs associated with faster running speeds and relatively shorter forelimbs preventing the latter from interfering with hind limb stride frequency. Accordingly, scansorial species are expected to have relatively shorter limbs, with the fore and hind extremities of more equal length, to promote the maintenance of their center of gravity closer to the substratum. Alternatively, terrestrial species in closed habitats could benefit from relatively shorter limbs because these may reduce their entanglement with more frequently encountered obstacles, whereas scansorial species could benefit from longer limbs which would result in greater limb spans and static stability. *Cyrtodactylus* is a speciose and ecologically diverse gekkonid genus. From generalist ancestors this clade has repeatedly produced specialists with narrow spatial niches, such as the occupancy of karst formations, caves, granite boulders, and different strata within the arboreal niche. For cave specialists, distinct morphological changes are associated with niche occupancy. The degree of morphological diversification exhibited by the other habitat specialists remains unknown, however. We investigated whether associations exist between limb dimensions and microhabitat use in this genus, to test if either of the opposing hypotheses can be confirmed for this successful radiation. To do so we measured body length and relative limb dimensions in a large sample, including all major lineages, reconstructed the phylomorphospace occupied by the species and tested for associations between spatial niche and limb morphology. We found strong separation between spatial niche groups, although overlap exists particularly among functionally related niches such as those of granite and karst specialists.

Oral presentation

Comparative 3D studies of pancreas morphogenesis in lizards of two clades: *Lacerta agilis* (Lacertoidea) and *Lepidodactylus lugubris* (Gekkota)

Rupik W.^{1,*}, Kaczmarek P.¹ Kowalska M.¹

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The pancreas is an important organ involved in digestion and carbohydrate metabolism. The morphology of this gland varied depending on phylogenetic position and species. The morphology of the pancreas in lizards is only poorly known. This study was focused on the development of the pancreas in two species of lizards belonging to two distinct clades: *Lacerta agilis* (Lacertoidea) and *Lepidodactylus lugubris* (Gekkota). Embryonic tissues were fixed and stained for light microscopy. Then, 3D reconstructions of pancreatic tissue with surrounding organs on the base of serial histological sections were performed. The pancreas of both lizard species was initially developed from three buds that were distinct located. During development, these buds fused, and the pancreas formed a central body connected with the intestine from which three branch out. The shape of the gland was bulbous. One branch (splenic lobe) was directed toward the splenic anlage and located near the spleen. The second one, located most anteriorly, was connected with the gall bladder. The third branch was localized between the loops of the intestine and situated most posteriorly. During embryonic development, the pancreatic lobes of both species become larger; however, the pancreas of *Lepidodactylus* remained bulbous. Moreover, the pancreatic branchings of *Lacerta* have become very elongated. The anterior pancreatic lobe of *Lacerta* was directed to the gall bladder towards the anterior part of the body. Moreover, the anterior pancreatic lobe of *Lepidodactylus* was curved and directed to the spleen anlage, near the splenic lobe. In both species, the gall bladder was located far from the pancreatic anlage. The splenic lobe was not in contact with the spleen. Differences in pancreatic morphology of studied species can be related to their phylogenetic position. It could also be associated with the shape of the body cavity. The study was supported by NCN grant (OPUS) - 2019/35/B/NZ4/00905.

Oral presentation

A novel geometric morphometric approach to identify snake prey vertebrae from raptor bird nests

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The Hungarian meadow viper (*Vipera ursinii rakosiensis*) is an endangered species, which faces high predation pressure, partially due to bird species that feed in its habitat. One of the best ways to measure the degree of pressure caused by them is by examining their diet. In order to achieve this, we developed a novel geometric morphometric approach to identify vertebrae of the snake species found in the Hungarian meadow viper habitats (*V. u. rakosiensis*, *Natrix natrix*, *Coronella austriaca*). We used linear discriminant analysis fitted on a reference material of vertebrae of identified snake species, and we tested the efficiency of the identification approach on presacral and caudal vertebrae with simulation levels of damage (missingness of landmarks). Parallely, we identified vertebrae of unknown species of snakes obtained from nests of short-toed eagles (*Circaetus gallicus*) and common buzzards (*Buteo buteo*). The accuracy of identification was more than 90% in the case of presacral vertebrae above wholeness of 75%. We had identified the vertebrae obtained from bird nests as bones of *N. natrix* (n=63, 85%), of *C. austriaca* (n=2, 3%) and of *V. u. rakosiensis* (n=9, 12%). Only the 2 *C. austriaca* vertebrae and one *V. u. rakosiensis* vertebra were originated from common buzzard nests, while the rest of the remains were found in or around short-toed eagle nests. Based on the attributes of the involved predator and prey species, the high ratio of *N. natrix* vertebrae seems ecologically feasible. This novel method could facilitate further research to examine the diet of predatory birds to discover trophic networks and predation pressure on species of conservation interest.

Anatomy and morphology

Oral presentation

Vertebral regionalization vs. morphological integration in *Lissotriton* newts

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Serially homologous structures, such as the vertebral column, often undergo functional and evolutionary diversification and are a good model-system for studies of regionalization and morphological integration. We studied these topics in the vertebral column (atlas, trunk and sacral vertebrae) of the closely related taxa of small-bodied newts – *Lissotriton schmidtleri*, *L. vulgaris ampelensis*, *L. v. meridionalis* and *L. v. vulgaris*, using 3D geometric morphometrics on models that were acquired with micro-CT scanning. Two different statistical approaches were employed to test for vertebral regionalization and overall morphological integration, namely segmented linear regression (SLR) and a partial least squares method (PLS). We observed a common pattern of regionalization, with a transition point after the 5th trunk vertebra. It corresponds with the antero-posterior transition common for tetrapods. Morphological integration, assessed via PLS analysis, is strongest at the 6th and 7th trunk vertebrae, while the anterior and distal parts of the vertebral column are less integrated. The PLS analysis of the asymmetric component of shape variation revealed a weak integration, statistically significant only among subsequent trunk vertebrae. In summary, the vertebral column of the closely related *Lissotriton* newts is subtly regionalized, while being morphologically integrated overall. There is a complex relationship between regionalization and morphological integration of the vertebral column, most likely influenced by the newt's bi-phasic life cycle that instigates different functional constraints in the aquatic and terrestrial life stages.

Anatomy and morphology

Oral presentation

Diet and habit explain head-shape convergences in natricine snakes

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The concept of ecomorphs, whereby species with similar ecologies have similar phenotypes regardless of their phylogenetic relatedness, is often central to discussions regarding the relationship between ecology and phenotype. However, some species have been grouped as ecomorphs based on phenotypic similarity without demonstrating ecological similarity. Within snakes, similar head shapes have convergently evolved in species living in comparable environments and/or with similar diets. Therefore, ecomorphs could exist in some snake lineages, but this assertion has rarely been tested for a wide-ranging group within a single framework. Natricine snakes (Natricinae) are ecomorphologically diverse and currently distributed in Asia, Africa, Europe and North-Central America. They are primarily semiaquatic or ground-dwelling terrestrial snakes, but some are aquatic, burrowing or aquatic and burrowing in habit, and may be generalist or specialist in diet. Thus, natricines present an ideal system to test whether natricine snakes from different major habit categories represent ecomorphs. We quantify morphological similarity and disparity in head shape among 191 of the ca. 250 currently recognised natricine species, and apply phylogenetic comparative methods to test for convergence. Natricine head shape is largely correlated with habit but in some burrowers is better explained by dietary specialism. Convergence in head shape is especially strong for aquatic burrowing, aquatic and terrestrial ecomorphs, and less strong for semiaquatic and burrowing ecomorphs. The ecomorph concept is useful for understanding natricine diversity and evolution, though would benefit from further refinement, especially for semiaquatic and burrowing taxa.

Anatomy and morphology

Oral presentation

Insights from the inside: 3D embryonic development of *Triturus newts*

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Large-bodied newts (*Triturus* sp.) represent a good model system for evo-devo studies. Females lay relatively large eggs, which are protected by transparent jelly layer, enabling easily observation of external anatomical features throughout embryonic development – from fertilized egg to fully formed larvae. MicroCT scanning aids another, more in depth insight into the development. Virtual cross sections enable visualization of internal anatomy, while volume renderings and reconstructions of 3D models enable exploration of development of internal organs and external morphology. Throughout *Triturus* embryonic development, homozygous embryos die due to a balanced lethal system known as chromosome 1 syndrome, which involves two non-recombining types of chromosome 1 (1A and 1B). It is hypothesized that the two potential homozygous genotypes (1A1A and 1B1B) arrest at different embryonic stages and express different phenotypes. Hence, knowledge of the embryo's genotype is crucial for understanding normal embryonic development of heterozygous embryos and malformations which lead to lethality in homozygous embryos. In a pilot project, we tested and optimized a protocol for *Triturus* embryo scanning which allowed exploration of external and internal morphology while preserving the DNA. Our protocol provides an opportunity to further explore embryonic development in *Triturus* in diverse research lines: 1) normal embryonic development as a base line for further studies; 2) arrested development to uncover the basis of lethality; and 3) testing various evo-devo hypotheses.

Poster presentation

Changes in thyroid histomorphology and thyroglobulin immunostaining upon exposure to endocrine disruptor thiourea in *Triturus newts*

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Aquatic ecosystems receive the majority of global pollution. Therefore, aquatic organisms are the most exposed and the most vulnerable as penetration of various endocrine disruptors is easier, resulting in high bioavailability and bioaccumulation of chemicals. One of the potent endocrine disruptors (anti-thyroid agents) is thiourea, which chemically blocks the synthesis of thyroid hormones and prevents metamorphosis in amphibians. The mechanism of action is very similar for all anti-thyroid agents via inhibition of thyroid peroxidase enzyme (TPO), thereby inhibiting iodination of tyrosine residues in thyroglobulin, the oxidative coupling of iodinated tyrosine and therefore the biosynthesis of the thyroid hormones. We investigated the influence of two non-lethal concentrations of thiourea (0.05% and 0.1%) on histomorphology of the thyroid gland in *Triturus newts* at the metamorphic stage, when TH concentrations should reach maximum level. Exposure to thiourea induced hypertrophy and hyperplasia of follicular cells as well as a significant reduction ($p < 0.05$) of interstitial tissue. Intensity of thyroglobulin immunostaining significantly decreased ($p < 0.05$) upon both thiourea treatments. Additionally, we tested mammalian thyroglobulin antibodies for immunohistochemical assessment of the thyroid follicular tissue in newts. Successful cross-reactivity of human primary antibody in immunochemical detection of thyroglobulin in *Triturus newts* in this study confirms potential homology in protein structure throughout the vertebrates.

Poster presentation

Craniofacial development of *Triturus* newts – a suitable model system for testing the developmental hourglass model

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In developmental biology a plethora of studies support the existence of a conserved stage during the ontogeny of vertebrates – the pharyngula. The developmental hourglass model predicts that the most conserved morphological pattern occurs in the middle of embryonic development. We explored external morphology of the craniofacial region of *Triturus* newts using 3D geometric morphometrics. The craniofacial region was selected because it experiences pleiotropic developmental constraints due to its major role in feeding, respiration and the housing of substantial parts of nervous and sensory systems. We aimed to uncover differences in variance between successive developmental stages, because it is proposed that the more constrained stages should have the lowest variance. In addition, we explored differences in the craniofacial shape between successive developmental stages. Stages included start from the moment after the completion of neurulation and span the mid-tailbud phase. The least amount of variance in shape was recorded at stage 24 (according to D'Amen and colleagues). In general, this stage is characterized by changes in overall head shape, the distinction of optical vesicles, intensive somitogenesis and formation of the tailbud which overgrows the blastopore, indicating that these processes could be highly constrained. Only significant craniofacial shape change occurred between this and successive stage. The most pronounced differences were due to growing of the head region and gill bud balancers. Optic vesicles became concave due to inductive processes preceding eye formation. These preliminary results indicate that stage 24 could be the most conserved one during early ontogeny as it is preceded and followed by stages exhibiting more variance in shape. Our results indicate that the craniofacial development represents a suitable model system for testing the hourglass model of development. Larger sample size, exploration of the entire embryonic development and ontogeny of additional newt species is needed to confirm our preliminary findings.

Poster presentation

Agriculture transformation affects development in the Iberian asp viper as reflected by high levels of fluctuating asymmetry in the head shape

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The transformation of the landscape due to intensive agriculture leads animals to use habitats with sub-optimal conditions (e.g. shelter scarcity) and/or increased stressors (e.g. agrochemicals). Research developed in oviparous reptiles has shown that deviation from the optimal conditions during egg incubation can destabilise embryonic development, leading to increasing levels of Fluctuating Asymmetry (FA, i.e. bidirectional small random deviations away from symmetry that is normally distributed and centred in zero). However, how environmental stressors derived from habitat alteration affects the development of non-laying egg reptiles remains scarcely addressed. In this work, we used Iberian populations of the asp viper, *Vipera aspis zinnikeri*, as a model to test if landscape transformation to intensive agriculture affects phenotypic variation related to development in a viviparous reptile. We used landmark-based geometric morphometrics to quantify variation in dorsal head shape across 128 individuals sampled in both intensive agriculture and natural, well-preserved areas in north-eastern Spain. We tested for the presence of FA using a Procrustes ANOVA, and we calculated an individual unsigned asymmetry index (uAI). Then, we compared the uAI of 67 individuals from intensive agricultural habitats and well-preserved natural habitats using Generalized Linear Models to also take the population structure of our sampling into account. We observed significantly higher FA levels in animals from intensive agricultural habitats than in those from well-preserved natural habitats. Our results indicated FA as a good bioindicator of developmental stress in viviparous reptiles, also warning of the potentially negative impact that habitat transformation to intensive agriculture may play during gestation and/or post-natal growth in this species.

Poster presentation

Limbs anomalies in the newt assemblage from Western Carpathian: a micro-CT approach

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We decided to assess the prevalence of limb anomalies and biomechanical adaptation of pathological limbs in four newt species that occur sympatric (*Triturus cristatus*, TC; *Ichthyosaura alpestris alpestris*, IA; *Lissotriton montandoni*, LM; *Lissotriton vulgaris vulgaris*, LV). We analyzed 1,781 individual newts preserved in alcohol from the collection of the Sarisske Museum Bardejov, Slovakia. The sample was collected during the breeding season from 1958 to 1980 in north-eastern Slovakia, around Bardejov in Western Carpathians. The individuals with limb anomalies were scanned using a micro-CT using the phoenix v|tome|x s device. The type of limb anomaly and biomechanical properties of the limb bones were assessed. The most common limb malformations were polydactyly and forked finger, while other abnormalities, including syndactyly, were the rarest. The hind limbs were more affected than the front limbs (25 vs 11 cases; only one newt had both simultaneously). The frequency of anomalies in each species' was as follows: IA - 4.76%, LM - 1.79%, LV - 0.88% and TC - 0%. The analysis of biomechanical properties is still ongoing. The frequency of anomalies in the sample for all analyzed species was within the range observed in other amphibian populations. The high frequency of abnormalities in LM and IA may result from their ecology and frequent colonization of very small water bodies (like temporary puddles and ditches), where aggressive behaviour may occur more often, resulting in limb injuries. TC and LV occur in larger reservoirs; therefore, aggressive behaviour or exposure to predation seems to be lower. A more significant proportion of males with limb pathologies may result from a more extended stay within the mating sites in large aggregations, which increases the risk of injury from conspecific.

Anatomy and morphology

Poster presentation

Comparative 3D developmental studies of gekkotans - lessons from the mourning gecko *Lepidodactylus lugubris* and leopard gecko *Eublepharis macularius* (Squamata, Gekkota)

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Depending on the group of reptiles, the pancreas of adult specimens is characterized by different morphology. The pancreas of lizards comprises three limbs: one is formed from the dorsal pancreatic bud, and two others are formed from the ventral buds. Among lizards, differences in pancreas morphology are associated mainly with length and degree of fusion of pancreatic limbs, distance from the spleen anlage, and size of the so-called juxtasplenic body. The development and morphology of the embryonic pancreas in lizards are poorly known. This study focused on the embryonic structure and morphology of the pancreas in two species representing Gekkota, mourning gecko *Lepidodactylus lugubris* and leopard gecko *Eublepharis macularius*, during the latest developmental stages. Embryonic tissues were fixed and stained for light microscopy using standard methods. 3D reconstructions of pancreatic tissue with surrounding organs were performed based on serial histological sections. At the end of embryonic development, the pancreas of the studied species possessed three branchings connected in the pancreas body. One of them (splenic limb) was located near the spleen anlage and formed a small thickening near the spleen – the juxtasplenic body. The second one was placed more anteriorly nearer the gall bladder anlage, and the third one was located between the intestine loops. Interestingly, the anterior limb in both studied species was curved and directed to the spleen near the splenic branching. Splenic branching in *Eublepharis* was located at a smaller distance to the spleen than in *Lepidodactylus*. A large accumulation of pancreatic islets was found within the splenic branch. In *Lepidodactylus* the juxtasplenic body was larger than in *Eublepharis*. The similar morphology of the pancreatic anlage in the studied species could be related to their phylogenetic proximity. The study was supported by NCN grant (OPUS) - 2019/35/B/NZ4/00905.

Poster presentation

Squamate lung morphogenesis – a lesson from mourning gecko *Lepidodactylus lugubris* (Squamata, Gekkota)

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The adult squamates such as lizards and snakes possess paired unicameral lungs. Their lungs are localized in the pleuroperitoneal cavity, and they are attached by a dorsal mesopneumonum. Moreover, the surfaces of the lungs in squamate have been enlarged by the development of the interconnected folds into a series of various heights their exchange. The morphogenesis of lungs in reptilian species has been poorly understood. For this reason, the aim of this study was the structural and 3D analysis steps of the lung differentiation in the mourning gecko (*Lepidodactylus lugubris*). Embryos used for this study were derived from professional breeding. Females of *L. lugubris* glued eggs to the vertical surfaces of the terrarium. The eggs were left and incubated in the terrarium at approximately 23-30°C. The embryos of the *L. lugubris* were isolated and collected a few times a year. The developmental stage of the embryos was evaluated based on the specific developmental table. Embryonic tissues for light microscopy were fixed, cut, collected, and staining using standard methods. 3D reconstructions of the lungs with surrounding organs were performed on the base of serial histological sections. Based on the histological analysis and 3D reconstructions, it was found that the stages of differentiation of the respiratory system of the studied species do not differ from those described in other Tetrapoda. The lungs represent the simplest type and the epithelium lining the wall of the them is formed by two cell types. The squamous cells located on the blood capillaries, is similar as type I pneumocytes of the other reptiles. Moreover the cuboidal cells located within capillaries can be named pneumocytes of type II. However, the 3D reconstruction shows that the left lung differentiates earlier than the right. Shortly before hatching, the left lung is wider and longer than the right one.

Poster presentation

Embryonic development of the skull in a parthenogenetic model organism, the mourning gecko (*Lepidodactylus lugubris*)

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Despite the long history of studies on the skeletal development in squamates, many groups of these hyperdiverse clade are still understudied. One of such groups are the gekkotans, a species-rich clade of mostly nocturnal lizards, encompassing limbless and fully-legged, terrestrial and arboreal species. This clade occupies also a crucial position in the squamate tree of life, being one of the earliest-branching lineages. Unfortunately, studies on the cranial development in this important clade concerned mostly the development of the chondrocranium, while the osteocranium was studied in fewer species and, in most cases, only in a few selected stages. We studied the development of the bony skull in the mourning gecko (*Lepidodactylus lugubris*), a species of small, arboreal gekkonid. It is a parthenogenetic, all-female species which recently emerged as a model parthenogenetic vertebrate. We double-stained (using mostly the non-acidic staining method) 55 embryos spanning the entire embryonic bony skull development. Several additional embryos were thin-sectioned and studied histologically. Unsurprisingly, the first bone to appear was the pterygoid, followed closely by the prearticular and supraangular. Other early-ossifying bones are the dentary, frontal, parietal and squamosal (although the ossification of the latter two is barely noticeable at first). Ossification around the time of hatching is relatively poor, with a large parietal fontanelle. The posterior margin of the parietals begins to ossify shortly before hatching. Also, the fusion of the braincase bones occurs postnatally. The ossification sequence is similar to most other hitherto studied squamates, but it is noteworthy that the bone-bearing premaxilla and maxilla ossify relatively late in *Lepidodactylus lugubris*.

Session: Biodiversity conservation



Pelobates balcanicus

Biodiversity conservation

Oral presentation

Experimental reinforcement of Hermann's tortoise populations impacted by fire

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Urbanization, habitat fragmentation and wildfires represent major threats to Mediterranean reptiles. Populations of the Hermann's tortoise (*Testudo hermanni*), a long live species, pay a particularly heavy death toll. This is the case for the main continental populations in France: more than half of the National Natural Reserve of the Maures (RNNPM, 5.276 hectares) was consumed by flames in 2021 and huge number of tortoises died. Without practical actions, the persistence of tortoise populations is compromised. Reinforcement of decimated populations with captive individuals is a straightforward option. Several prerequisites must be considered however: 1) Are burnt areas suitable to host both surviving and translocated tortoises? 2) Will translocated tortoises settle in their new habitats and effectively contribute to population reinforcement? Long-term telemetric monitoring and field experiments provide encouraging responses. Various proxies (e.g. survival rate, behavior, body condition, eco-physiological traits) clearly suggest that translocation is a valuable technique to help fragile populations. Ongoing experiments further evaluate the usefulness of juveniles (3-8 years old) originating from breeding programs ("head started") to reinforce another population that was severely impacted by a fire in 2017 in a natural reserve 25 km south of RNNPM.

Biodiversity conservation

Oral presentation

Strict nature reserve status and snake population maintenance

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Intensive agriculture and sprawling urbanization are the main causes of habitat and biodiversity loss. Strictly protected areas aim to stem such alarming trends. Yet, strictly protected temperate forests are subjected to progressive closing, further squeezing bushy habitats that are essential to many reptiles. Since 1990's, long-term mark-recapture study of snake populations were launched in the forest of Chizé (Western-central France) situated in a landscape ravaged by industrial and intensive agriculture. During more than 25 years, snakes were searched using a network of >1,000 corrugated slabs. Snakes were measured, sexed, marked and released at their exact place of capture (1,624 whip snakes, *Hierophis viridiflavus* and 3,169 Aesculapian snakes, *Zamenis longissimus*). Recaptures (N=1,486 and N=1,836 respectively) enabled us to perform demographic analyses. During the first decade of the study, logging of plots generated a mosaic of contrasted habitats (e.g., open meadows, old forest patches). In 2006, almost all the study area (>2,600 ha) was classified as strictly nature reserve (Integral Biological Reserve, category 1a IUCN). This status bans forest management and promotes the growth of trees at the expense of bushy habitats. This situation offered a quasi-experimental framework to investigate the impact of differential forest closure across space and time on snake populations. We used demographic model framework (with U-care, E-surge and MARK) to estimate detection and recapture probabilities, population sizes, transience, survival and recruitment rates. Our results show that habitat closing and the disappearance of open bushy habitats provokes a sharp decline of snake populations. Applying the most protective conservation status to forest sanctuaries might be less valuable than a protective status where conservation management actions are permitted.

Biodiversity conservation

Oral presentation

Diversity, distribution and conservation of the West African Crocodile in Mauritania

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The West African Crocodile (*Crocodylus suchus*) once ranged across the Sahara-Sahel, but increasing aridity and recent human persecution have extensively fragmented desert populations. In Mauritania it was known from c.24 localities, but given their remoteness, there is relatively scarce knowledge about population status. Crocodiles have been surveyed in southern Mauritania since 2008, during 14 overland expeditions. We provide data on the distribution, occupied habitats, population size and structure, landscape connectivity, threat factors, and conservation actions for crocodiles in Mauritania. The presence of crocodiles was confirmed in 93 localities, with an additional 10 likely localities, 10 transient localities, and 3 of confirmed extinction. Over 80% of these 116 wetlands are mountain rock-pools, river beds, and floodplains. In 55 surveyed wetlands, most contained less than 10 individuals and 20% contained only one individual. Molecular markers uncovered genetic differentiation patterns and a metapopulation structure related to hydrographic basins. Northern populations displayed higher levels of genetic isolation, while southern populations showed signs of population admixture and gene flow. Water indexes suggested putative corridors along seasonal rivers and a potential link between dispersal/gene flow and water availability. Patterns of water connectivity in 2010s and 1980s (representative of regional drought) scenarios suggested that dispersal is likely mediated by networks established during the rainy seasons. The 1980s drought may have severely impacted population connectivity. Eleven IUCN threat factors were identified, being droughts and temperature extremes, water abstraction for domestic use, and nomadic grazing the most frequent. Future research is aimed at estimating population structure using genetic and genomic data based on expanded sample datasets, and simulating climate change impacts in habitat suitability and population connectivity. Sustainable development in local communities and promotion of wetland biodiversity conservation are needed. A system of micro-reserves connected by river beds can be used to protect biodiversity.

Biodiversity conservation

Oral presentation

Facultative paedomorphic newts in the Balkans: hotspot, habitats, declines, and threats

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Facultative paedomorphosis is a polyphenism that implies the development of two alternative adult phenotypes: the metamorphs that lose their gills at metamorphosis and the paedomorphs, which retain them. Our aim was to depict their patterns of distribution, habitats, declines and threats across the Balkans with the support of multiple local collaborations. Metamorphosis is the most common developmental process in newts and one of the main hotspots for newt paedomorphosis occurs in Balkans with the three genus involved: *Ichthyosaura*, *Lissotriton* and *Triturus*. In Alpine newts, the largest populations of paedomorphs were described in an area located between Montenegro and northern Greece, occupying mountain lakes where population sizes can reach thousands of individuals, but also present in lower numbers in mid-elevated ponds and wells. Paedomorphic Greek smooth newts and Macedonian crested newts were typically associated with ponds. Although the former constituted sometimes large populations, the second was usually represented only by a few individuals. Other paedomorphic populations were also described in other Balkan countries. However, recent surveys evidenced high population losses in most habitats, particularly in Montenegro where the situation is catastrophic with almost all populations of paedomorphic newts now extinct, reducing the current hotspot. Paedomorphs from several emblematic subspecies also vanished in several Balkan countries. The main environmental driver of the declines was the introduction of fish, and more recently crayfish. Based on Corine land cover, land use had no significant effect on population losses but care should also be taken to future changes of land use. An increase of urbanisation and pollution was indeed found at the border of some lakes and some habitats became inadequate for newts. There is therefore an urgent need to stop fish introductions and to reverse habitats to more pristine pre-fish conditions to maintain the little dragons of the Balkans.

Biodiversity conservation

Oral presentation

Successful translocation of moor frog (*Rana arvalis*) and pool frog (*Pelophylax lessonae*) in the Netherlands

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Amphibians suffer under the growing pressure of economic development in western Europe. The destruction of their habitat is one of the main causes for declining populations. For protected species, habitat loss needs to be compensated by law through the creation of new habitat. However, an evaluation of these compensation measures in the Netherlands showed that the applied measures are often inadequate to sustain the affected amphibian populations. These failures are either due to a lack of ecological knowledge when the measures are designed or due to the poor execution of the measures in the field. Additionally, after realisation of the compensatory habitat, there is often a lack of management of the site, which results in a low success rate. In this presentation we highlight how a successful compensatory habitat for moor frog (*Rana arvalis*) and pool frog (*Pelophylax lessonae*) can be constructed and we show how successful translocation of these species to this new habitat was executed. In the centre of the Netherlands, near the city of Nieuwegein, a new business park has been developed on extensively managed agricultural land. In 2016, in order to compensate for the loss of habitat, new habitat for both species was created on former agricultural land approximately two kilometres to the north. The new habitat includes key elements such as breeding habitat, land habitat and hibernation sites. Habitat development was monitored and management was adjusted to create and sustain favourable conditions. Moor frogs (adults and eggs) and pool frogs (adults) were translocated for multiple years between 2016 and 2019 from different development sites. Furthermore, other rare and protected species have been translocated or compensated by the newly created habitat including the European weatherfish (*Misgurnus fossilis*), spined loach (*Cobitis taeneo*) and bitterling (*Rhodeus amarus*). Monitoring by counting eggs, (calling) adults and larvae was carried out between 2016 and 2021. By 2021 we could confirm that there are established breeding populations of moor frog and pool frog.

Biodiversity conservation

Oral presentation

The impact of road traffic on the reptiles and amphibians in the Nature Park “River Zeta”

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Road infrastructure is an integral part of the development of local communities as well as entire regions. It enables the connection of people, strengthens the economy and makes the system more efficient. At the same time, road infrastructure and traffic are associated with water, air and soil pollution, and have one of the biggest impacts on the reduction of animal populations. Animals die either in direct contact with vehicles or indirectly because the road creates a physical barrier that prevents them from accessing the breeding areas, feeding grounds, and shelter. Amphibian road-kills were monitored over a five-year period in the Nature Park “River Zeta” on the one low and one high traffic volume road, with a total length of 30 kilometers. The total number of recorded road-killed individuals during 5 years of monitoring is 3,098, out of which 1,538 are amphibians and 1,560 are reptiles. The most affected species by the traffic in this Nature Park are: *Bufo bufo*, *Emys orbicularis*, *Testudo hermani* and *Malpolon insignitus*. Thanks to these data, the first culverts for amphibians, reptiles and mammals were made in Montenegro, right in the Nature Park “River Zeta”.

Biodiversity conservation

Oral presentation

Conservation practices of yellow-bellied toad and midwife toad in The Netherlands

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The natural distribution of yellow-bellied toad (*Bombina variegata*) and midwife toad (*Alytes obstetricans*) is limited to the south of The Netherlands. From the 1960's there was a dramatic decline in the distribution of yellow-bellied toad. Midwife toad distribution remained stable, though population densities went down. The main cause of decline for both species was the disappearance of small cattle ponds. In 1982 an action plan was launched to preserve and restore amphibian populations in Southern Limburg by restoring and constructing more than 500 ponds. For both species this only had a temporary effect due to a lack of management and unsuitability of many ponds for reproduction of yellow-bellied toad. In 2001 only five locations with yellow-bellied toad and 12 locations with midwife toad remained and a national monitoring program started for both species. In this program adult and subadult yellow-bellied toads are counted three times a year and tadpoles of midwife toad are counted once a year. A breeding programme for yellow-bellied toad started in 2005, followed by reintroduction of the species at an additional five locations. Monitoring showed a strong increase in the population trend from 2006 until 2012 followed by a decline since 2013. Conservation measures like artificial cart tracks and basic habitats like small swamps turned out to be susceptible to overgrowing and drying up. To overcome this, more than 200 concrete cattle troughs were dug into the ground in 2018. They are more easy to manage and less vulnerable to drying up. Currently yellow-bellied toad occurs at 19 locations and populations are more and more connected. At the same time midwife toad faces local extinction, though overall numbers of tadpoles are increasing. More habitat restoration for both species is planned in the future to achieve a favourable conservation status.

Biodiversity conservation

Oral presentation

Czech herpetofauna monitoring and mapping

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Mapping and monitoring of habitats, plants and animals is very important for nature conservation in Czechia. And this has been case of batracho- and herpetofauna too, especially since 2008. We have a vast national database of all species, 380,000 amphibian and reptile records, from which 275,000 originated from the last 15 years.

There are several sources of the data. The most important is an extensive systematic mapping of every grid (2.5 x 3 km, KFME based) at least once in every 6 to 10 years and important site surveys. The second systematic survey is the species monitoring at selected sites. These data are supplemented by evaluated citizen science data. The data from other independent surveys and publications enrich the database, too.

The subject of monitoring are Habitat Directive species, 15 out of 21 amphibian taxa and 6 out of 13 reptile species at important sites, especially SACs. The scheme aims at species presence confirmation, abundance estimate and successful reproduction evidence. Standard methodology is very important; e.g. newt trap using is crucial in case of *Triturus* species monitoring because results gathered are much better than the previous ones.

The current status of species is well known. We pay attention not only to native ones but also to alien *Trachemys scripta* and also newly discovered *Podarcis tauricus*. The most common amphibians are *Bufo bufo* and *Rana temporaria*, while the rarest are *Lissotriton helveticus*, *Triturus carnifex* and *dobrogicus*. Among reptiles, common are *Lacerta agilis* and *Natrix natrix*, while rare are *Podarcis muralis* and *Emys orbicularis*.

Our data can be easily displayed using map applications, which are accessible for authorities, as well as to the public. Only in some species e.g. *Zamenis longissimus* they are sensitive and not public. They are used in national Red List assessment, important sites management and in conservation planning.

Biodiversity conservation

Oral presentation

Application of occupancy modeling framework to inform conservation of a small terrestrial turtle

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The Ornate box turtle (*Terrapene ornata*) is a widely distributed North American Emydid turtle but listed as near threatened by the International Union for Conservation of Nature due to habitat destruction, pet trade, and road mortality. *Terrapene ornata* is a small and secretive species, which can pose a challenge to conducting systematic surveys and assessing species conservation status. Occupancy models have become an important analytical tool for assessing species-habitat relations while explicitly accounting for imperfect detection. Our objective was to assess the feasibility of applying 'space-for-time substitution' occupancy modeling framework to *T. ornata* transect line survey data in the Great Plains of New Mexico, USA, to better understand which environmental variables affect species presence. From August to October 2018, we surveyed 34 sites across three different habitat types, where each transect line within a site represented a spatial replicate. Our study suggests that species detection was most influenced by individual observers and the time of day. We also found that *T. ornata* occupies habitats with less dense ground cover, but avoids highly altered habitats (i.e., cultivated fields). This study is the first to apply transect line surveys of box turtles to occupancy modeling framework. Despite several limitations, our study design suggests that this approach can be applied to small terrestrial turtle surveys to obtain broad generalizations about species occupancy over large-scales and provide information on species-habitat relations which can aid conservation measures.

Biodiversity conservation

Oral presentation

IUCN Red List assessment of the Western Mediterranean vipers, *Vipera latastei* and *Vipera monticola*. New perspectives derived from a taxonomic update

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The International Union for Conservation of Nature (IUCN) provides specific means to evaluate the conservation status of biodiversity components – species, ecosystems, etc., i.e. Red Listing. For many taxa, factors as reduced information on species natural history or taxonomic uncertainties have hampered the Red Listing assessments or their update. The Western Mediterranean vipers *Vipera latastei* and *Vipera monticola* have recently been object of a taxonomic update, implying important distributional rearrangements and the definition of three subspecies within each species. Here we follow IUCN Red List guidelines to assess the conservation status of both taxa at the global level and the regional levels of Spain, Portugal, and Morocco, also considering their subspecies. We analysed distributional and environmental data on species and subspecies occurrence using Geographical Information Systems and Ecological Niche Models to calculate IUCN metrics and assess their range fragmentation. We also used published and non-published demographic and reproductive information retrieved for four populations of *V. latastei*, information from other published studies, and ad hoc observations on habitat transformation gathered for more than 20 years of fieldwork campaigns. Both taxa are identified as Vulnerable at the global and regional levels, *V. latastei* according to the criteria of population size reduction (A2) and geographic range (B2), *V. monticola* to the geographic range criterion. Worryingly, two subspecies of the North African species, *V. monticola monticola* and *V. monticola atlantica* are considered as Endangered following the geographic range criteria. Climate change and landscape transformation are identified as the major threats affecting the conservation of both species and their habitats. Conservation management must be assisted by population studies that provide key demographic and ecological information, and monitoring population trends in the upcoming years. In addition, the conservation of *V. monticola* requires the development of basal research on distribution, particularly for some subspecies as *V. monticola atlantica*.

Biodiversity conservation

Oral presentation

Dwarf vipers come out big: perspectives on distribution, taxonomy, and conservation of grassland vipers in West Asia

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There is a guild of three phenotypically similar dwarf vipers of steppes and grassland from Western Asia (Turkiye, Transcaucasus, Iran), all with a threat status assessed by IUCN 2009: *Vipera anatolica* (CR), *V. renardi eriwanensis* (VU) and *V. darevskii* (CR). As their distribution and ecology is little known, and even their systematics stands with many uncertainties, meaningful conservation strategies are negatively affected. Ten years ago, we begun to develop a database by sorting out literature records, social media accounts, and acquiring new records from our and other team's field expeditions. Based on a minimum distance of 1 km between two locations to reflect an individual small viper's home range, we compiled > 300 single locations of dwarf vipers between Turkiye, Transcaucasus (area adjacent-south of Greater Caucasus), and Iran. New morphological analysis of partly sympatric (but not syntopic) *V. renardi eriwanensis* (incl. *V. r. shemakhensis* and *V. r. ebneri*) and *V. darevskii* (incl. *V. sakoi*) shows a few consistent, easy to detect interspecific differences. For *V. sakoi*, we prefer a subspecific status due to medium *cyt b* divergence of 3–5% to *V. darevskii*, extreme phenotypic and ecological similarity, and massive lack of geographic sampling between them. The enlarged dataset on dwarf vipers permits also some finer environmental distinctions between *V. renardi eriwanensis* and *V. darevskii*, although data resolution is still too coarse for traditional SDM to pick up the often small-scaled macrohabitats that *V. darevskii* inhabits. Clear environmental delineation of each dwarf viper taxon may require direct on site data recording. This updated dataset will make new threat assessment for the three dwarf vipers species necessary, despite much of their relevant biology still remains unknown.

Oral presentation

Distribution and preliminary population size of the freshwater turtles (*Emys orbicularis* and *Mauremys rivulata*) on the Montenegrin coast

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The freshwater habitats of the Mediterranean are one of the most vulnerable habitats in Europe where some of the most endangered species live, including the Balkan terrapin and European pond turtle. These habitats have been shrinking for the last 50 years, and their area is rapidly decreasing over the last 20 years due to unsustainable tourism, sewage, and concreting of watercourses. Certain populations of freshwater turtles on the Montenegrin coast have disappeared because the streams where they lived were completely concreted.

That is why we explored detailed distribution of freshwater turtles, and estimated the size of their populations in 4 locations: Igalo (Municipality of Herceg Novi), Jaz beach and Buljarica (Municipality of Budva), and Tivat.

During 5 years of monitoring 4 populations of both species at 4 locations, 2,282 *Emys orbicularis* and 820 *Mauremys rivulata* individuals were recorded and marked.

According to preliminary data, the size of the *Emys orbicularis* population in the Igalo is over 600 individuals, in the Tivat also over 600, and in the Jaz over 700, while in the Buljarica there are over 2000 individuals. The population size of *Mauremys rivulata* in the Igalo is over 300 individuals, in the Tivat also over 300, in the Jaz is over 350 and in the Buljarica is over 300 individuals.

The results show that there is a large anthropogenic impact due to the expansion of urban areas and the increased amount of waste waters. Discharge of huge amount of unprocessed waste waters leads to the accelerated growth and eutrophication of wetlands. That is why urgent conservation measures and further research (especially in Buljarica) is necessary. The research of these populations will continue in order to define the applicable measures for the protection of these species and their habitats as well as their proper management.

Biodiversity conservation

Oral presentation

LIFE Integrated Project for Enhanced Management of Natura 2000 in Slovenia – An expert basis for Amphibian Conservation in two selected Natura 2000 sites

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Our study is part of the LIFE-IP NATURA.SI project (LIFE17 IPE/SI/000011, 2018–2026). The project predominantly aims at enhancing the management of Natura 2000 in Slovenia in cooperation with key sectors (e. g. nature protection, agriculture, forestry) and other stakeholders. All of them should be aware of available approaches and best practices in nature conservation and be able to implement them on a national, systemic level, bearing in mind the local environments. There is a general lack of systematic amphibian research in Natura 2000 sites in Slovenia. Therefore, two Natura 2000 sites were selected as an example of how local species conservation status should be assessed. In ‘Slovenska Istra’ the focus species are *Rana latastei*, *Triturus carnifex*, and *Bombina variegata*, and in ‘Ličenca pri Poljčanah’ the focus species is *B. variegata*. The first-ever thorough surveys of the areas were conducted in 2019 (app. 90 field-trip days, 80 km² of surveyed area), and the monitoring of the selected locations will be carried out until 2026. The conservation status of all three species in ‘Slovenska Istra’ in 2019 was assessed as unfavourable (insufficient aquatic habitats and their poor quality), while the conservation status of the yellow-bellied toad in ‘Ličenca pri Poljčanah’ was favourable (good connectivity of many aquatic habitats and suitable terrestrial habitat). A number of threats have been identified: loss of aquatic habitats due to drought and human, pollution of aquatic habitats with fertilizers and plant protection products, presence of introduced fish and non-native turtle species in standing waters. Direct and systemic measures to improve amphibian habitats and their populations have been recommended, moreover Natura 2000 Management Programme (PUN 2022–2028) is going to be effectively updated. In order to improve and maintain favourable conservation status of all three species, it is crucial to successfully implement long-term systemic measures.

Biodiversity conservation

Oral presentation

Toads in trees and spots on frog. Using citizen science tools to learn new things about well-known amphibians

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New technology and combining novel monitoring methods with citizen science can substantially improve our understanding of species trends, conservation mitigation or ecological traits. We used collaborations and large-scale data searches with major citizen science projects surveying for arboreal mammals in Great Britain to investigate potential tree climbing by amphibians and separately, we tested and validated a novel capture-mark recapture for common frogs (*Rana temporaria*) using smart phones to facilitate monitoring by public members. We found widespread arboreal usage by amphibians in England and Wales and especially common toads (*Bufo bufo*), of arboreal nest boxes and tree cavities but the reasons for this remain unknown. Of the 277-400 sites surveyed annually for arboreal mammals since 2009 at least 18 sites had amphibians recorded in nest boxes while of the 1388 trees surveyed for bats 1.4% had toads present. Intensive monitoring of common frogs in 2018-2022 in suburban East Anglia revealed constant survival rates but a clear pattern of activity across all 12 months of the year due to rapidly disappearing hibernation. Novel citizen science tools can demonstrate surprising gaps in knowledge of species ecology for otherwise well studied species.

Biodiversity conservation

Oral presentation

An evidence-based approach to conservating amphibians and reptiles in Europe

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Conservation literature is increasing rapidly in volume but complex barriers remain in place that restrict its practical implementation value, the relevance for and access by practitioners as well as the usage in the field. Evidence-based approaches can substantially improve conservation transparency and effective learning and represent the first step in generating cost-effectiveness assessments of multiple potential conservation actions that are available for a given problem. Using global reviews of evidence synthesis for amphibians and reptiles I highlight progress to date as well as knowledge gaps and I discuss the inclusion of synthesis products into decision making via the use of conservation guidance and simple but standardised cost reporting frameworks. This includes 1086 reviewed and synthesized studies across 441 conservation actions for over 350 species. I will also discuss future evidence synthesis opportunities and tools using artificial intelligence approaches for automation, dynamic meta-analysis and decision support tools.

Oral presentation

Influence of overgrowing processes in sandy deserts and semi-deserts on the abundance and species diversity of reptiles

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The inhabitants of sandy deserts and semi-deserts in different regions of the world found themselves in a situation of serious biocenotic changes. Sand overgrowth process and the associated reduction of characteristic habitats is taking place everywhere. First, it concerns reptiles. The negative consequences of the biotope overgrowing for its inhabitants are the deterioration of the conditions of movement and hunting and decline of communication within the population. These changes adversely affect not only typical psammophiles, but also relatively eurybiont species. The reduction of characteristic habitats leads to a decrease in the number of reptiles up to the complete disappearance of typical psammophiles.

Our ten years study (2010-2019) on three species of lizards in the semi-deserts of the Lower Volga region: multicolored lizard (*Eremias arguta deserti*, Gmelin, 1789), spotted toad agama (*Phrynocephalus guttatus guttatus*, Gmelin, 1789) and toad-headed agama (*Phrynocephalus mystaceus mystaceus*, Pallas, 1776) made it possible to describe the mechanism of lizard adaptations to the reduction of the characteristic biotope. At the beginning of sand overgrowth, the migration activity increases up to 53% in search of suitable habitats. Then, most of the individuals concentrate on optimal habitats. This leads to overcrowding of groups, and the mechanisms of autoregulation of numbers begin to work. The result is a reduction of approximately 63% in the number of settled groups and a decrease in the size of the population as a whole. The overgrowth of ecological corridors blocks the flow of migrants necessary for the growth of isolated settlements and for the gene pool exchange within the population. The further process of overgrowth leads to the disintegration of groups and disruption of the mosaic distribution of individuals over the territory. In typical psammophiles, to which *Phrynocephalus mystaceus mystaceus* belongs, after mass migrations, the population rapidly disappears from the altered territory.

Biodiversity conservation

Oral presentation

Effects of different grassland management regimes on the abundance of the endangered Hungarian meadow viper (*Vipera ursinii rakosiensis*)

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The Hungarian meadow viper lost most of its habitats and the remaining ones are exploited for economic interests. Grazing, mowing and switching between these managements are the three typical grassland utilization methods, however, their effects on the quality of meadow viper habitats were not studied before. Therefore, we first conducted monitoring of vipers in nine 100×100m plots in differently managed habitats in spring and autumn of 2019. We followed this up with a second, fine-scale monitoring study in 78, 50×50m plots in spring and autumn in 2020 and 2021 mostly on grazed habitats, also with measuring the productivity of the pasture and including information about herding style. We estimated viper density using n-mixture modelling. The first sampling showed a positive effect of grazing management on viper density, while mowing and switched usage had significant negative effects. The second, fine-scale sampling showed that herding style had no significant effect, while grazing pressure -which interacts with grassland productivity- had significant negative effect on viper density. This suggests different carrying-capacity among plant associations. Our results suggest that a change from mowing to grazing is vital for the wellbeing of Hungarian meadow viper habitats, and the reduction of grazing pressure is recommended, since extensive grazing creates more favourable conditions for the species.

Biodiversity conservation

Oral presentation

Updating knowledge on the herpetofauna of an endangered ecosystem: the Vjosa river (Albania)

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Within the Balkan region many rivers are threatened with dam constructions, causing irreversible habitat destruction and placing species at risk. One such river is one of Europe's last wild rivers, the transboundary Aoos/Vjosa river. The construction of several hydropower plants (HPP) are proposed along its catchment area; 31 of which are anticipated within the Albanian region. As one of Europe's least explored rivers, limited scientific research has been conducted along the Aoos/Vjosa, creating a significant knowledge gap of its biodiversity. To increase our knowledge, a rapid survey was conducted in May 2019 (12th – 19th), contributing to the Vjosa project organised under the "Save the Blue Heart of Europe" campaign, led by RiverWatch and EuroNatur NGO's. Seven sites were surveyed, six of which threatened by Poçem and Kalivaç HPP projects, for species presence and abundance, with a primarily focused on species listed in Appendix II of the Habitats Directive. Species identification was determined by using field guides, the prior knowledge of observers and photos taken for confirmation for the most challenging species groups (*Lacerta*, *Pelophylax*). Six amphibian and 13 reptile species were recorded during the survey, comprising of 79 records. The majority of these records are species of International and National conservation interest (three globally assessed as near threatened; four enlisted Appendix II of the Habitats Directive; 14 enlisted Appendix II of the Bonn Convention; 10 assessed as threatened in the Albanian Red List).

The construction of HPP dams presents a significant threat for the species recorded in our survey areas of Poçem and Kalivaç. At particular risk is the potential loss of the side river arms and karst resurgences areas, which provide supporting habitats for aquatic turtles and breeding grounds for amphibian species.

Biodiversity conservation

Oral presentation

The right of passage: Nature corridors for the amphibians and reptiles in Romania

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Land-use changes, decrease of landscape heterogeneity and increased fragmentation are recognized as leading causes for the loss of biodiversity, locally viewed as even more important than climate changes. The consequences of such activities are loss of species, alteration of faunal assemblages and changes in the ecological processes. One of the key challenges in such areas of intense anthropogenic pressure is preserving the remaining patches, on which communities ultimately depend for survival. Historically, nature conservation has relied on areas of various sizes managed as nature reserves for the conservation of species, but today there is a consensus that this is not enough and the entire landscape needs management through habitat corridors that facilitate dispersal and genetic exchange. Amphibians and reptiles are perfect models for habitat connectivity modeling as a result of their sensitivity to environmental changes and limited dispersal abilities, but are often avoided because little information is available on their biology and ecology. We employed connectivity modeling based on resistance surfaces to test whether the Natura 2000 sites could be used as core areas to be integrated within a true ecological network for the 46 species of amphibians and reptiles in Romania within a 50-year timeframe and using only remaining natural features. Results showed no connectivity for *Vipera ursinii rakosiensis* and reduced connectivity for *Triturus dobrogicus*, *Bombina bombina*, *Vipera ursinii moldavica* and *Vipera [berus] nikolskii*, while *Vipera ammodytes ammodytes* had perfect connectivity. Mapped corridors provided connectivity for up to 27 species at a time and the regions best connected were in the Carpathian Mountains or along the lower Danube River. Pooling together the least-cost paths revealed previously unknown important areas for the connectivity of amphibians and reptiles, located outside the boundaries of designated protected sites.

Biodiversity conservation

Oral presentation

Integrating citizen science with grass snake conservation: six-year results of an online management tool for grass snake breeding heaps in the Netherlands

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The distribution of the barred grass snake (*Natrix helvetica*) in north-western Europe is historically linked with human settlements (livestock husbandry) and especially with the presence of compost and manure heaps, from Neolithic times up to the 20th century. These man-made breeding sites are utilized for oviposition and incubation, and are often more effective for successful breeding than natural sites in this part of Europe. However, during the past century breeding sites have largely disappeared, driven by a shift towards large scale industrial agriculture and more stringent environmental legislation; and, whereas traditionally every livestock farm would have a manure heap, such features are now few and far between. These major changes have had a significant impact on the number and availability of suitable breeding sites for these egg-laying snakes and may contribute to population declines. To redress this issue, NGOs, land managers, allotment associations and citizen scientists in the Netherlands have been creating grass snake breeding heaps. RAVON has supported this effort by creating an online platform (www.broeihopen.nl) where the volunteer citizen scientist “grass snake working groups” can create their own account, enabling them to enter data such as the location of breeding heaps, heap composition, number of (un-)hatched eggs and observations of grass snakes. Egg counts can be visualized in a graph comparing the number of hatched vs. unhatched eggs for a specific heap, but also across all heaps. Since the creation of the platform (2016) around 80 working groups have joined the scheme reporting > 200,000 eggs which are submitted to the National Database of Flora and Fauna. This online platform can provide an effective tool to educate, engage and connect volunteers in order to conserve and restore the historic close bond between humans and grass snakes as a companion species.

Biodiversity conservation

Oral presentation

Establishment of ecological networks in Serbia – field research of batracho- and herpetofauna

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The establishment of ecological networks (including Natura 2000) is one of the priorities in the conservation of important species and habitats in the Republic of Serbia. During the last three years (2020-2022), we conducted field research on amphibian and reptile species listed in the Annexes II & IV of the Habitat Directive. The main goals were to obtain data about precise geographic distribution, estimate population statuses and conservation threats, as well as to propose conservation measures for the studied populations and their habitats. Field studies lasted more than 70 days and included over 60 locations across the country. Focal groups were species listed in Annex II: *Triturus cristatus*, *T. dobrogicus*, *T. ivanbureschi*, *T. macedonicus*, *Testudo graeca*, *T. hermanni*, *Emys orbicularis*, *Elaphe quatuorlineata* and *Vipera ursinii*. Results of these studies provided a more precise geographic distribution of crested newts and their hybrid zones in Serbia. For two species, we proposed the protection of two locations at the national level: one in eastern Serbia (for *T. ivanbureschi*) and one in southern Serbia (for *T. macedonicus*). The most threatened regions, where massive habitat losses and changes were detected, are the valleys of Velika and Zapadna Morava rivers, as well as wetlands along the Danube and Sava rivers. Populations of crested newts in Serbia are endangered by several threatening factors: habitat destructions/alterations, agriculture, water pollution and climatic changes. For reptiles, the most important locations for conservation of species listed in Annex II were detected in southeastern, southern and southwestern Serbia. For three reptile species with limited distribution and small populations, we proposed two locations in southern and southwestern Serbia for protection at the national level. The main threats for those species are habitat destruction/alternation and climatic changes. Results of these studies pointed out inconsistencies in conservation priorities between the national and European levels.

Biodiversity conservation

Oral presentation

At the forefront of conservation: integrating science and amphibian reintroductions in Flanders, Belgium

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In Flanders, Belgium, several amphibian species are declining. Loss of -, and decreasing quality of habitats are the main drivers. To reverse this trend, species-specific action plans have been implemented by the government. Currently, reintroduction efforts are ongoing for common midwife toad *Alytes obstetricans* and common spadefoot toad *Pelobates fuscus*. Both species have been lost from much of their former range in Flanders, warranting the use of reintroductions as a last resort to restore populations and attain a favourable conservation status. Next to these two projects, a population of great crested newt (*Triturus cristatus*) was translocated following an ex-situ breeding program. Captive breeding (*A. obstetricans* and *T. cristatus*) and headstarting (*P. fuscus*) methods have been optimized, congruent with supporting research focused towards increasing larval growth and survival in an effort to maximize reintroduction success. The aforementioned reintroduction programs follow an integrated approach, combining genetic data and habitat restoration with translocations and post-release monitoring. While the reintroduction programs are still in the early stages, we report on the successes, setbacks and pitfalls of our efforts.

Oral presentation

Predicting mortality risks of colubrid snakes on Cyprus roads using habitat suitability modelling

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The direct and indirect impacts of extensive road networks on wildlife is widely acknowledged and studied. Aiming towards both improving road planning/management and assisting wildlife conservation, large databases and citizen science initiatives have been put forward in the last few decades. For the case of Cyprus, the Cyprus Roadkill Observation System (CyROS) is such an initiative, launched in 2017, and currently holding more than 1.000 records. This paper reports on the extent of the phenomenon observed in the herpetofauna of Cyprus, using racer snakes (Colubridae family), the most frequently reported roadkill in Cyprus as a case study group. The volume of available information was spatially analysed in R through multi-level Habitat Suitability Modelling (HSM) framework to identify areas with high probability of roadkill occurrence throughout the island. This framework uses habitat suitability indices (HSI) from coarser resolution level (1km², including land cover, temperature, precipitation, road density) as an explanatory variable in models at the finest resolution level (100m², including distance from land cover types, distance from wetlands/rivers/urban areas), allowing the downscaling of HSM. This approach provides insights on species response at the local level. We discuss mortality risk in relation to the type of roads (e.g. highways and main/secondary roads) and the adjacent landscape and emphasize the urgency of acting retrospectively, to minimize fragmentation and conserve biodiversity.

Biodiversity conservation

Poster presentation

Comparing two approaches for a long-term monitoring of the great crested newt (*Triturus cristatus*) in the Grand Est region, France: site occupancy vs. N-mixture models

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While a decline of great crested newt (*Triturus cristatus*) populations is well documented in some regions of France, its status in the newly created Grand Est region (north-eastern France) is not precisely known. A long-term monitoring program has been initiated in 2021 in order to assess its abundance variation over time through a randomised sample of 172 ponds within nine natural regions where the species is found. Two different statistical models have been confronted back-to-back, one solely based on occurrences (site occupancy model), and another integrating multiple counts at each site (N-mixture model), with the aim of obtaining two indicators to track trends of the species at a local and global scale. We found an occupancy rate of 0.73 associated with a detection probability of 0.76 with the first model, and a total population estimated at 1414 newts with a detection probability of 0.30 with the second model. We discuss the core differences between the two approaches, from the sampling design to the model construction, and focus on potential limits associated with these modelling techniques, particularly when applied to newts. This comparative study should allow to improve the efficiency of the long-term monitoring of this endangered species.

Biodiversity conservation

Poster presentation

Disappearing archosaurs – an assessment of established protected areas in the Philippines to save the Critically Endangered and endemic philippine crocodile *Crocodylus mindorensis*

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Once distributed all over the Philippines, the endemic Philippine Crocodile *Crocodylus mindorensis* is threatened with extinction. Less than 140 mature individuals live in the wild. Human activities like fishing and illegal poaching, as well as land-use change and habitat conversion cause a continuing threat for the remaining populations. Therefore, designated protected areas were evaluated with species distribution models (SDMs) for improved future conservation efforts. For this purpose, the existing IUCN-reserves were analysed for climatical suitability (combining bioclimatic and remote sensing variables), wetland occurrences and the human footprint index by using MaxEnt and QGIS. Based on topical species records, our final SDM-model showed high performance and revealed the climatically most suitable areas on Luzon and Mindanao. However, only small parts of the climatically optimal area and suitable wetlands are currently covered by reserves. Case in addition, none of the species' records is located within a protected area. The anthropogenic pressures in the reserves were diverse and varied between a low and moderate level. Most of the records are found in areas with a moderate to medium human footprint. Considering the three criteria, only 'Northern Sierra Natural Park', 'Agusan Marsh Wildlife Sanctuary' and 'Upper Agno River Basin Resource Reserve' revealed to be suitable conservation areas for *C. mindorensis*. The other reserves seemed to be unsuitable, whereas suitable areas outside protected areas are highly recommended for further surveys. Therefore, the current network of existing protected areas needs significant improvement to provide well-suited and long-term protection for *C. mindorensis*. More surveys are also necessary to find hidden, so far overlooked populations and verify *C. mindorensis* tolerance level for human footprint.

Biodiversity conservation

Poster presentation

Monitoring of European pond turtle using remote techniques

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Determining population density and regularly monitoring certain populations can be very time consuming and sometimes, not very efficient. For those reasons, we examine the efficiency of monitoring of turtle population in several ways, without the need to catch the animals in order to identify each individual. In the first part of our research in the Nature Park Kopački rit, in Croatia, we applied standardized method of catching European pond turtle, *Emys orbicularis* with the use of hand nets and traps. In total, 16 individuals were caught, and each caught individual was measured, sexed, photographed and marked with unique temporary mark, and afterwards released in the same place where it was caught. In the second part of our research, the same area was photographed using drone, and separately using tele-photo-lens, and the individual turtles recorded were compared to those from the first part of our research. Besides identification of already caught and marked individuals on photos obtained either from drone or by hand-held camera, we also used pattern of yellow marks on the head as a way of individual recognition. Application of distance monitoring of pond turtles, such as those examined in our research, should become a wide-used practice, since it does not require significant amount of time and effort, and also does not disturb the animals.

Biodiversity conservation

Poster presentation

Contribution to amphibian conservation on a high traffic road in central Ljubljana, Slovenia

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Most amphibian species require terrestrial and aquatic habitats for their life, reproduction, and development. Especially in spring some species migrate from their terrestrial and hibernating sites to their spawning sites. The migration paths are often crossed by roads and the mortality of amphibians on such sections can be very high. One such road section with high amphibian mortality is located in the capital of Slovenia, Ljubljana. Amphibians hibernate in forests south of the road near ZOO Ljubljana, while their spawning sites are mostly located across the road in the ZOO and nearby forest. There are eight amphibian species occurring at this location and the most common are: the common toad (*Bufo bufo*), the common frog (*Rana temporaria*), and the agile frog (*R. dalmatina*). Amphibian mortality was first surveyed here in autumn 2007 and spring 2008 when results identified a high mortality rate during spring migrations. In 2008, the Herpetological Society – Societas herpetologica slovenica, began an annual conservation action to protect amphibians by putting up a temporary fence at one side of the road during spring migrations. These activities have been repeated every year since 2008 and have two main objectives: i) informing the general public about the importance of amphibian conservation, and ii) gathering data about the number, species composition and exact locations of amphibians crossing the road. We looked at temporal variation of amphibian migrations and tested how the temperature and precipitation affect their migration using the complete and precise data available from the past 9 years (2014 – 2022). By monitoring the population size of species found in that area and determining the exact locations of crossing, we can contribute to the knowledge on future permanent conservation measures.

Biodiversity conservation

Poster presentation

Monitoring of amphibians in Podyjí National Park, Czech Republic

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The Podyjí National Park is a relatively small area (92 km²) in the forested Central European landscape, situated along a deep river valley. Amphibians breed here mainly in small fishponds and various types of pools; almost 100 localities of this type have been identified in the area. Additional pools have been systematically built since 2000, some of the fishponds have a controlled fish stock. Amphibians have been studied in the national park since the 1990s, and in the last 10 years a systematic monitoring has been carried out at 38 localities. The results of monitoring show that typical forest species of amphibians dominate (*Bufo bufo*, *Rana dalmatina*, in some parts of the area also *Rana temporaria*) and their populations seem to be stable over the observed period. A decrease in the number of localities and population size was recorded in species inhabiting non-forest wetlands, shallow littorals and waterlogged meadows (*Hyla arborea* - slight decrease, *Bombina bombina* and *Rana arvalis* - marked decrease). On the contrary, an increase was proved in species with a rapid response to pool building and targeted fishpond management, especially in *Triturus carnifex*, which is the protected phenomenon of the local Natura 2000 site. Thanks to the inspection of the vast majority of localities suitable for reproduction, we can estimate the current abundance of some species – the local populations of *Bufo bufo* and *Rana dalmatina* have about 5,000-10,000 adults, in *Hyla arborea* they are probably lower hundreds of adults, while *Bombina bombina* and *Rana arvalis* reach only lower tens of adults in the area. Despite the atypical environment for amphibians, the Podyjí National Park is their important refuge in the cultural landscape of South Moravia.

Biodiversity conservation

Poster presentation

Five years of monitoring amphibian and reptile populations at National Park „Kopaonik“, Serbia

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National parks are, by definition, areas set aside for wildlife protection, but also human recreation. Sometimes, the development of tourism-related infrastructure and capacities or resource exploitation can impede the primary function of national parks – nature conservation. Baseline and trend monitoring are great tools to assess human pressure on nature. Amphibian and reptile species have small ranges and are vulnerable to changes in their environment which makes them great bioindicators. Here we present the results of five-year monitoring activities of amphibian and reptile populations at National Park „Kopaonik“, Serbia. Data on batracho- and herpetofauna of NP „Kopaonik“ were limited and outdated, so a detailed survey of species richness, habitat conditions, and population trends was greatly needed. Starting from 2018 to 2022 we described the amphibian and reptile diversity of the national park and systematically monitored several habitats, dispersed throughout all three protection zones as well as outside protection zones, to assess the habitat quality and population trends of selected species. We had an opportunity to see the effect of the international travel ban (due to the Covid19) on batracho- and herpetofauna, when much more people spent their vacation at NP „Kopaonik“ than usually. Per our results so far, the richness of the amphibians and reptiles species of the NP „Kopanik“ is moderate (9 amphibian and 12 reptile species). The population size of the selected lizard species is stable through the years regardless of the protection zone. Amphibian populations are also stable, as offsprings of the majority of the species were found every year. However human pressure on the amphibian and reptile species is high. Habitat degradation is present and some localities are at great risk to be lost.

Biodiversity conservation

Poster presentation

Temporal effects of groundwater level on the occurrence and habitat suitability of the endangered Hungarian meadow viper (*Vipera ursinii rakosiensis*)

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Grassland habitats are among the most threatened ecosystems globally. In the current changing environment, drought and changes in groundwater levels are the most significant threats to these habitats besides habitat alteration. We studied the long-term and short-term effects of groundwater levels on the occurrence and habitat suitability of the endangered Hungarian meadow viper (*Vipera ursinii rakosiensis*, HMV) by answering the following questions. What is the trend and degree of change of groundwater at the Kiskunság habitats of HMV? What is the effect on the elevation of HMV occurrence records of groundwater level? Is there seasonal variation in the elevation of HMV occurrences? What are the temporal and spatial effects of groundwater levels on HMV habitat suitability? The study area covered the viper habitats in the Kiskunság region where we used data from 9 groundwater monitoring wells between the years 1931-2021, and also we analysed data from 10 wells at Felső-kiskunsági turjánvidék collecting data between 2020-2021. Changes in groundwater levels followed an upward and downward trend over the nearly 100 years studied, but declining periods were more common. There was a significant relationship between the relative altitude of the HMV occurrence points and the groundwater level estimated at the point of occurrence, and it was characterized by a seasonal pattern. In summer, low groundwater levels had a negative impact on habitat suitability. The stability of the groundwater level-based habitat suitability index was significantly higher in areas where HMV occur than in other parts of the study area. Based on the results, the availability of grasslands exposed to excess water is important for HMV, so most likely the drought in Kiskunság area will have a negative effect on its population. Decreases in groundwater levels could be mitigated or offset by interventions for active and adaptive water retention.

Biodiversity conservation

Poster presentation

Last chance to see? Iran and India as strongholds for the Marsh crocodile (*Crocodylus palustris*)

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Predictions of future change in species distributions are necessary for defining adequate conservation planning actions over space and time. The Marsh crocodile (*Crocodylus palustris*) is native to the freshwater habitats of the Indian subcontinent and in south-eastern Iran. Habitat loss is currently the most important factor threatening crocodile dispersal and persistence, and climate change will likely place increasing pressure on populations. This study used ecological niche modelling (Maximum entropy) to predict the current distribution of the species and to project it to future climate conditions. For this purpose, 380 occurrence records were used for model computation and environmental data were obtained from Worldclim 2.0. Averages of eight global circulation model outputs assuming four IPCC6 per story lines in 2081-2100 were used as future ensembles. Furthermore, future possible anthropogenic pressure was quantified using economic growth models. Temperature Annual Range was the climatic variable with highest contribution in the modelling. Presently, the most potential suitable habitats are located in Sri Lanka, south-eastern peninsular of India, tropical moist forest along the west coast of India, border between Nepal and India, and the south coast of Iran and Pakistan. In the future, these suitable habitats are predicted to be further fragmented and to move further inland. Additional threats may arise due to increased human conflicts due to human population growth. Conservation actions should therefore focus on those areas which remain climatically comparatively stable with a low potential of human conflicts. Key areas are located in the northern parts of India and at the westernmost range edge of the species in Iran.

Biodiversity conservation

Poster presentation

Patterns of snake road mortality in a complex landscape with different flood regimes

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Flood control measures can have a large impact on ecosystems, habitats and species but the comparison of active floodplains versus flood-protected areas are rare. In our research we compared road mortality patterns of two semi-aquatic snake species, *Natrix natrix* and *N. tessellata* in roads neighbouring a regularly flooded and a flood-protected landscape with similar, but complex habitat types and traffic volumes. The study took place in North-eastern Hungary where we monitored 58 km of roads in every two weeks in three years with bicycle, recorded the coordinates of snake carcasses and identified their age and sex if it was possible. Then, we investigated temporal and spatial patterns of road mortality in the flooded and flood-protected areas. We found a total of 1655 *Natrix* snakes and there were six times more snakes killed in the flooded then the flood-protected area. Mortality patterns were similar in the two species and had two peaks annually, one in late spring and early summer and one in autumn in both areas. Spatial pattern of mortality was more predictable from year to year in the flooded area but was influenced by similar habitat types in both areas. Urban green areas, arable land and other, mostly human dominated habitat types had usually negative effects, while woodlands, rivers and more naturalistic types had usually positive effects on mortality. Traffic volume, and the sex and age of snakes showed no effects on mortality. The resulted patterns are suggesting that snakes are usually killed when they are moving out from their hibernacula in spring and then again when they are moving back to winter because they cannot overwinter in flooded areas. To prevent road mortality, we suggest a series of underpasses and artificial hibernacula before the road.

Poster presentation

Microplastics in pond water and tadpoles of three toad species

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Microplastics (MPs) are an emerging threat to aquatic animals. However, impact, bioaccumulation and prevalence of these anthropogenic particles are not understood well enough. Microplastics were extracted from tadpoles of *Bufo bufo* (N=124), *Bufo viridis* (N=120), *Epidalea calamita* (N=117) and surface water collected from ponds (N=11) inhabited by tadpoles. MPs were scrutinized under a microscope at magnification of 40x and categorised on a basis of their morphology into fibres, fragments, flakes and granules. Validation and qualitative analysis of selected particles was carried out with the use of FT-IR ATR spectroscopy. 0.39% of tadpoles had injured tail. Most of them were in the 27th Gosner stage of development. Mean MP load per 1 tadpole of *B. bufo*, *B. viridis* and *E. calamita* equated to 4.315, 3.327 and 2.7 respectively. The most frequent morphological types of MPs were fibers (N= 676), fragments (N= 397) and flakes (N= 127). MP pollution of pond water ranged between 2 and 18.57 pieces per litre. In that matrix the most prevalent MP morphological type was a fragment. Spectroscopy of MPs from tadpoles turned out 19 materials, 13 of which are plastics. The most frequently detected materials were, in descending order, poly(propylene:ethylene:diene), polyethylene, and polypropylene. The most frequently detected non-synthetic material was cellulose. FT-IR ATR measurements of the water samples detected 9 materials, 5 of which are plastics with the most common being polypropylene.

Biodiversity conservation

Poster presentation

Drones as a novel technique to estimate the population size of the Galápagos Marine Iguana

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The marine iguana (*Amblyrhynchus cristatus*) is an endemic species of the Galápagos Archipelago. The IUCN Red List of Threatened Species classifies it as vulnerable to extinction due to increasing threats, principally: invasive species, pollution, climate change, and urbanization. The marine iguana is an iconic well-studied species; however, a reliable complete population size estimate is not available, mainly because monitoring it with traditional methods (ground-based surveys) is logistically difficult, dangerous, and simply impossible for some colonies (e.g. on cliffs). Technological advances in recent years allowed drones to emerge as an efficient and safe alternative to monitoring wild populations, especially for threatened or hard to reach species. We report here our efforts to test the use of drones for marine iguana monitoring by undertaking aerial surveys and comparing the outcome to traditional ground-based surveys, performed in parallel. This work included four colonies, representing four subspecies, on three main islands. Aerial surveys involved drones flown mainly from boats, and ground-based methods were capture mark-resight (CMR) and simple counts. Results showed that the higher abundance registered for all sites was obtained with CMR, as expected, as this is a two-day survey that accounts for imperfect detection probability. However, between drones and simple counts (the method commonly used by wildlife managers), drones registered higher abundance (17-35%) on all sites, evidencing better outcomes with less effort and lower risk to surveyors. To reduce the workload of data analysis, we are currently testing citizen science and machine learning as potential approaches to crowd-source and automate counting. We will survey the entire archipelago by drone, whilst continuing to perform traditional surveys; the overall aim is to estimate the population size of all 11 subspecies from the aerial images. In addition, we are working with local wildlife managers to develop and implement the new method for future monitoring efforts.

Session: Biogeography and distribution



Lacerta viridis

Biogeography and distribution

Oral presentation

New Bufonids from understudied Angolan endemism centers

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Recently there has been an intensive resurrection of herpetological studies in Angola. The reestablishment of peace in the country, after decades of civil war, allowed for scientific expeditions, which revealed new country records, and the description of many new species. Bufonids are among the most speciose families in Angola, with 14 species recorded, representing ~11% of the country's amphibian richness. Recently collected material revealed undescribed diversity among two charismatic African endemic genera: Red toads, *Schismaderma*, and Pigmy toads, *Poyntonophrynus*. Integrative analysis using genetics, morphology, osteology, bioacoustics, and biogeographic data, lead to the identification of new *Schismaderma* and new *Poyntonophrynus* species. Each of them seem to be country endemics, and associated with particular centers of endemism: the *Schismaderma* from the Angolan Miombo Woodland, and the *Poyntonophrynus* from the Angolan Scarp Savanna and Woodland, and the Angolan Montane Forest-Grassland Mosaic. These descriptions indicate what probably can still be discovered concerning Angolan fauna, and highlight the importance of more intensive research.

Biogeography and distribution

Oral presentation

Mapping the amphibians and reptiles of Namibia

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Namibia is a largely arid country of 824,292 square kilometers straddling the Tropic of Capricorn in southwestern Africa. It supports at least 277 species of reptiles and 50 frog species. In preparation for the publication of a book on the herpetofauna of Namibia more than 40,000 museum and citizen science records were gathered, georeferenced and plotted, passing through five iterations to remove erroneous localities, confirm problematic localities and integrate reliable literature references. Maps produced show point localities but also use a modified niche modeling approach combined with personally informed field truthing to present estimated range maps that are far more accurate than those currently available. Recent taxonomic changes, as well as targeted sampling in border areas with South Africa and Angola have revealed the presence in the country of several previously unrecorded species and many discontinuous distributions have been shown to be artifacts. For genera with many hundreds of point localities (e.g., *Pedioplanis*, *Pachydactylus*, *Chondrodactylus*, *Trachylepis*) distributional data provide corroboration for previously proposed historical geographical barriers, such as the Kuiseb River, the Erongo Mountains and the Namib Dune Sea, but previously underappreciated areas of localized endemism have also been confirmed (e.g., the Huns-Orange Mountains and the Baynes-Otjihipa Mountains). As expected, lizards typically exhibit small distributions, with many taxa with large ranges having been shown to represent species complexes. Snake distributions are often extensive in both their latitudinal and longitudinal extent and cross both historical barriers as well as bioclimatic zones. With aquatic habitats limited in Namibia, frog diversity is highly concentrated in the northeastern Kavango and Zambezi regions of the country.

Oral presentation

Evaluating the impact of water holes during the covid-19 lockdown on herpetofaunal community in arid regions southern Morocco

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Water holes are a built underground cisterns used by local peoples in Moroccan arid regions to retain rainwater (called Matfia) and also other underground water tank used to deliver drink water to citizens. Unfortunately, these holes are also serious traps for wildlife and then a cause of accidental mortality, especially for amphibians and reptiles. In this work, we aim to identify the impact of these infrastructures on herpetofaunal community from southern Morocco and its implications for conservation. In the last decade, southern Morocco, especially Bas Drâa-Guelmim area has become the target of herpetological excursions of international naturalists. However, because the covid-19 confinement, international flights and herpetological excursions are interrupted during the whole season of 2020. In October 2020, we took advantage of this opportunity to examined more than 260 water holes in this area in order to quantify the mortality and species trapped per hole and per year. Trapped animals belonging in 3 species of amphibians and at least 24 species of reptiles (8 snakes and 16 lizards), a total of 140 animals (50 % already found dead; with an average of 0.6 animal/hole). *Hemorrhois hippocrepis* and *Psammophis schokari* are the most animals found trapped (respectively 20 % and 13 %) followed by *Stenodactylus mauritanicus* (14 % geckos), *Bufotes boulengeri* (10 % animals) and *Uromastix nigriventris* (7 %). Finally, based on data of this study, we identified area for conservation priorities for the future managements of herpetofaunal community in the studied regional hot spot.

Biogeography and distribution

Oral presentation

Evolutionary patterns in New Guinea small-eyed snakes (*Micropechis ikaheka*) inferred with museomic approaches

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Museum specimens are an invaluable resource for understanding many aspects of biology, including but not limited to, biodiversity, evolution, ecology and conservation. Many global regions, especially in the tropics, lack recent exploration, and, consequently few tissue samples exist for genetic analyses due to the way that museum specimens are preserved (e.g., formalin fixed). Recently, modern genetic techniques have been used to generate sequence data from museum specimens, allowing for previously unanswered questions to be addressed. The island of New Guinea is one of the most poorly studied regions of the world, despite being home to one of the largest rainforests globally and home to countless undescribed species. There are numerous New Guinean species for which museum specimens were collected only during the 19th and early 20th centuries. The New Guinea small-eyed snake, *Micropechis ikaheka*, is a medically important snake distributed across the island of New Guinea. *Micropechis ikaheka* has rarely been encountered by recent biologists and thus only a small number of freshly collected tissue samples are available. However, large numbers of museum specimens exist from across the entirety of their range. Using a combination of mitogenomes and ultraconserved elements generated from fresh and museum samples, and morphological data, we will present findings on the biogeographic patterns observed of diversity in *M. ikaheka*. We demonstrate that *M. ikaheka* has undocumented diversity throughout their range with deep genetic splits between lineages. Additionally, we provide growing support for the usefulness of museomic approaches for addressing species level evolutionary questions.

Biogeography and distribution

Poster presentation

Diversity of the herpeto- and batrachofauna of the Lovćen mountain, Montenegro: a review and update

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This paper unites literature data and new findings by the author about areal distribution and diversity of numerous and diverse herpeto and batrachofauna from the Lovćen region. Conservation status of each species, at nacional and international level, is given, also. Mt Lovćen rises from the Adriatic coastline, closing the long and twisting bays of Boka Kotorska and making the hinterland to the coastal town of Kotor, from the Orjen Mt. in the west, to the Cetinje and Crmnica valleys in the north and north-east. This region represents a hotspot of herpeto- and batrachofauna diversity with numerous endemic and rare species. 26 species of reptiles and 10 species of amphibians were registered in this area.

Biogeography and distribution

Poster presentation

Modeling the effects of climate change on the distribution and phenotypic diversity of European grass snake (*Natrix natrix*)

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Range shifts, habitat loss, and species extinctions have already been observed due to climate change. The diverse morphs of polymorphic species have divergent niches and thermal physiological optimums and respond differently to the changing climate. Here, we used a database on the distribution of European grass snake (*Natrix natrix*) phenotypes derived from different citizen scientists' data-sharing platforms to produce species distribution models (SDMs) to evaluate the effects of the XXIst century climate change on the distribution of the phenotypic diversity of the species. Our results have shown a northward distribution shift of the species and changes in the predicted phenotype frequencies across the species range. We found that not all phenotypes will be affected in the same way. For example, areas suitable for lighter phenotypes are predicted to expand and become more frequent due to climate change. Informing SDMs with phenotypic data when studying the effects of climate change is extremely useful as it allows us to understand how populations from different areas of the species' range will be affected.

Biogeography and distribution

Poster presentation

Niche differentiation in the relict, endemic spiny frogs *Allopaa hazarensis* and *Chrysopaa sternosignata*

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The relict, endemic taxa *Allopaa* and *Chrysopaa* are key elements of the Hindu Kush-Himalayan amphibian fauna and share an early-Miocene evolution, making them important proxies for the reconstruction of the Paleogene history of the Tibetan Plateau and the evaluation of respective modern scenarios of the development of the HTO. However, there is no much information on the distribution of these taxa. We here provide species distribution models for both taxa and test the hypotheses of niche differentiation. The average performance of our MaxEnt models was considered significantly better than random. They show, that both taxa are geographically separated with an allopatric distribution pattern, and that BIO8 (mean temperature of wettest quarter) was most important to the models. Further, our findings provide strong support for distinct niche divergence among *C. sternosignata* and *A. hazarensis*. The study contributes to the knowledge about the distribution of these species and provide basic information for guiding future management of them.

Biogeography and distribution

Poster presentation

Some characteristic data of reptiles in the protected area of Germia

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During the study period (April - October 2021), mainly during the daylight hours, we conducted field research for the purpose of compiling an inventory of herpetofauna in the protected area of Germia, which is located near the city of Prishtina. The study area is characterized by the dry rocky terrain, limited mountain areas, and open mountainous areas. In total, nine reptile species (*Podarcis muralis*, *Podarcis tauricus*, *Lacerta viridis*, *Ablepharus kittaibelli*, *Anguis fragilis*, *Testudo hermanni*, *Zamenis longissimus*, *Coronella austriaca*, *Vipera ammodytes*) were recorded during this research project. Methodologies and techniques used were based on previously published studies on the same topic. All recorded individuals were captured, photographed, and released into their natural habitats, without being subjected to any harm. Therefore, our results have shown and confirmed the presence of these species in the studied area, as well as the great importance of Germia for maintaining high local reptile diversity. Furthermore, our findings imposed the necessity to ensure the survival of these species by establishing a long-term reptile monitoring of this area and by applying appropriate conservation measures. Moreover, as this area is also a recreation place, the raising of citizens' awareness about the value of reptile diversity in the area of Germia is another future task, and proper education is necessary for minimizing possible human-reptile conflicts.

Poster presentation

Updated distribution of the endangered and highly specialised meadow viper (*Vipera ursinii macrops*) in Serbia

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The meadow viper is the smallest and most endangered European viper, mostly due to the high level of habitat specialisation, low dispersal ability and fragmented distribution. In the Balkan Peninsula, isolated populations of *Vipera ursinii macrops* inhabit alpine and subalpine meadows above 1000 m. Here we updated the distribution of the meadow viper in the Mokra Gora Mt., SW Serbia, and revised the conservation threats affecting this subspecies and its habitats. Field sampling effort totalled 25 field days in spring and summer 2020-2022. A total of 8 vipers were found, distributed by three 1x1 km grid cells. Ecological models (developed using Maxent) based on climate and land-cover variables identified 28 grid cells on Mokra Gora Mt. with suitable conditions for the occurrence of meadow viper. The most important environmental factors related to its distribution were maximum temperature of the warmest month, precipitation of the driest and warmest quarters, annual potential evapo-transpiration, and land-cover (moors and heathlands, natural grasslands). Local conservation threats (following IUCN standard threats) include the potential development of tourism and recreation areas, renewable energy (windmill farms), logging and wood harvesting, with associated developments of road networks, and habitat shifting and alteration (abandonment of traditional pastoral farming and climate change). The low number of individuals detected in comparison to other Balkan populations suggests very small local populations. Additional localities in Serbia were identified as suitable for meadow viper occurrence (e.g. Jadovnik and Javorje Mts.) and they should be targeted for future sampling. Long-term conservation of the subspecies at the Mokra Gora Mt. requires a better regulation of logging and building activities, in order to safeguard the currently occupied habitats. The region should be considered for classification as a protected area, given the occurrence of other animal and plant species, which might lead to better habitat protection.

Biogeography and distribution

Poster presentation

Amphibians and reptiles of the Nature Park “Ulcinj Salina”, Montenegro

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The Nature Park “Ulcinj Salina” is situated in the extreme southeastern part of Montenegro and covers the area of the former largest saltpans in the Mediterranean region. It is identified as Important Bird Area, Emerald Site, Ramsar Site and potential Natura 2000 site in Montenegro. After salt production had stopped a decade ago, a rather quick succession started, causing alternation in community structure in favor of non-salt tolerant species. So far, Ulcinj Salina has not been systematically surveyed for amphibians and reptiles and available data actually refer to a broader area of southern Montenegro. Here I present the results of batracho- and herpetofaunal surveys of Ulcinj Salina conducted during 2021. Five species of amphibians (*Bufo bufo*, *Bufo viridis*, *Hyla arborea*, *Pelophylax ridibundus* and *Pelophylax shqipericus*) and nine species of reptiles (*Testudo hermanni*, *Emys orbicularis*, *Lacerta trilineata*, *Podarcis melisellensis*, *Anguis graeca*, *Pseudopus apodus*, *Dolichophis caspius*, *Natrix natrix* and *Natrix tessellata*) were recorded. The greatest diversity of batracho- and herpetofauna was recorded along the northern parts of the Nature Park where the succession from saline to freshwater habitats was most evident. The most abundant but restricted to certain parts of the area were *P. ridibundus*, *E. orbicularis* and *P. melisellensis*. A monitoring procedure is proposed for *P. shqipericus*, *T. hermanni* and *E. orbicularis* as species of special conservation importance. Currently Ulcinj Salina presents a refuge for amphibians and reptiles of the coastal wetlands of southern Montenegro that are exposed to a variety of threats due to rapid urbanization and pollution. Since the revitalization of salt production and establishment of a sustainable tourism is planned in Ulcinj Salina, adequate mitigation measures and maintenance of certain basins and channels in current freshwater state are necessary to preserve amphibians and reptiles of this Nature Park.

Biogeography and distribution

Poster presentation

Diversity and biogeography of the New Guinea slender-toed geckos (Gekkonidae: *Nactus*)

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New Guinea and surrounding islands are a vastly understudied region of high diversity and endemism. The reptile fauna of New Guinea is biologically diverse, with large numbers of undescribed species. The slender-toed geckos of the genus *Nactus* – endemic to Oceania and the Western Indian Ocean – are a poorly studied group of lizards, especially in New Guinea. Until recently, the “*Nactus pelagicus* complex” was used as a comprehensive term to describe *Nactus* distributed across New Guinea and adjacent islands, with recent morphological work suggesting the recognition of twenty-four putative species. Although a detailed morphological assessment of *Nactus* species within the “*N. pelagicus* complex” was completed in 2020, there still remains substantial taxonomic confusion, partly because of poor diagnoses. Most taxonomic and systematics research on *Nactus* has focused on morphology, with no molecular studies incorporating dense sampling of New Guinean *Nactus*. Here, we provide the first comprehensive estimate of phylogenetic relationships in New Guinean *Nactus*. We will present molecular phylogenetic analyses and species delimitation results from mitochondrial nd2 and DArTseq datasets from ca. 300 New Guinean and Australian *Nactus*, and find evidence for multiple colonisation events to Australia, deep lineage diversity in Milne Bay Province (Papua New Guinea), and repeated evolution of miniaturisation.

Biogeography and distribution

Poster presentation

Hidden diversity and natural history of the secretive New Guinea worm-eating snakes, genus *Toxicocalamus* (Elapidae: Hydrophiinae)

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Toxicocalamus is a genus of diurnal, terrestrial or fossorial, oviparous, vermivorous snakes, endemic to the island of New Guinea and some of its satellite islands. These snakes generally exhibit highland or island distributions, in rainforest, hill and pre-montane forests, or montane grasslands, and are especially common in earthworm-rich highland garden systems. Most species are relatively small (< 600 mm), but one species exceeds 1.0 m SVL. Many species exhibit distinctive head-scutel fusion patterns that greatly aid in their identification. The genus was erected in 1896 by Boulenger with the type species *T. longissimus* from Woodlark Island to the southeast of the Papuan Peninsula of modern-day Papua New Guinea. During the remainder of the 19th Century additional species were described, both in *Toxicocalamus* but also in three other genera (*Apistocalamus*, *Pseudapistocalamus*, *Ultrocalamus*) that were eventually synonymised within *Toxicocalamus* by McDowell (1967, 1969). At the end of the 1960s, *Toxicocalamus* comprised nine species, and this number remained the same for forty years until a new generation of herpetologists discovered new species. Since 2009 a further eight species were described, and at least five new species are in process of description. We estimate that *Toxicocalamus* will soon contain at least 26 species, making this oft-overlooked genus of secretive serpents the largest terrestrial elapid genus in the Hydrophiinae and the largest terrestrial snake genus in Melanesia. *Toxicocalamus* is currently represented by 528 specimens in thirty collections worldwide.

Biogeography and distribution

Poster presentation

Surface amphibians in caves of Slovenia – overview of findings and their conservation importance

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Of the 20 amphibian species native to Slovenia, only the olm (*Proteus anguinus*), lives exclusively in subterranean waters, while the rest are surface species. Many of the latter utilize caves as seasonal or daily shelters or occur there accidentally. In this contribution, we gathered all the records of surface amphibians reported from Slovenian caves by compiling data from the CKFF and SubBioDB databases. We aimed to assess surface amphibian species occurrence and abundances in such sites, in what seasons they were observed, and whether caves can be regarded as important for their conservation. There are approximately 130 caves with records of surface amphibian species. Adults and/or juveniles of at least 12 species were recorded: *Salamandra salamandra*, *S. atra*, *Ichthyosaura alpestris*, *Lissotriton vulgaris*, *Triturus carnifex*, *Bombina variegata*, *Bufo bufo*, *Hyla arborea*, *Rana dalmatina*, *R. temporaria*, *R. latastei* and *Pelophylax* sp. Data from most sites are random observations by members of both institutions or cavers (e.g., Citizen Science) – altogether more than 100 observers and more than 60 written sources. The only systematic research was conducted in the cave Vranja jama, a hibernaculum for over 10,000 individuals of five species, mostly of *R. temporaria*. Because of its high conservation value, the cave is closed for public. The outcome of our study, i.e. the information on cave sites with surface amphibians, will be included in the national nature conservation information system, which will be established in years 2020–2024 under the EU co-funded project Life NarcIS (LIFE19 GIE/SI/0001619). This information system will serve as a common framework for all the publicly funded biological data and nature conservation documents, which should improve the implementation of legislation through more efficient data access.

Biogeography and distribution

Poster presentation

Reptiles of the Republic of Srpska (Bosnia and Herzegovina): diversity, distribution and conservation

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The territory of the Republic of Srpska covers northern and eastern parts of the geographical area of Bosnia and Herzegovina. As a continental entity within B&H, Republic of Srpska is characterized by significant habitat diversity (although there is no access to the sea), as well as rich biodiversity. The aim of this paper is to present the diversity of reptiles of the Republic of Srpska, based on the authors' field research and literature data. Field investigations in the given area were conducted between 2011 and 2021, when 26 autochthonous species of reptiles were recorded: two species of turtles (Chelonia), 11 species of lizards (Sauria), and 13 species of snakes (Ophidia). In addition to the species list documented by the field researches, according to literature data, the territory of the Republic of Srpska is inhabited by two more autochthonous lizard species. The reptiles of the Republic of Srpska are systematically arranged into 21 genera, 10 families and two orders. Regarding their zoogeographical remarks, they are grouped into nine chorotypes. The most species-rich chorotype are eastern Mediterranean (11 species) and South-European (five species). Two reptile species from the Republic of Srpska (*Vipera ursinii* and *Dinarolacerta mosorensis*) are considered as Vulnerable (VU) on the IUCN Red List, while three species (*Emys orbicularis*, *Testudo hermanni* and *Elaphe quatuorlineata*) are listed as Near Threatened (NT). Four reptile species (*Algyroides nigropunctatus*, *Dalmatolacerta oxycephala*, *Dinarolacerta mosorensis* and *Hierophis gemonensis*) are endemic to the Balkan Peninsula, and the presented data on their distribution may serve as a good starting point in defining the herpetologically important areas in the Republic of Srpska.

Session: Biology and evolution of behavior



Testudo hermanni

Oral presentation

Drop dead gorgeous: death feigning behaviour in three distinct colour morphs of dice snakes

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Prey animals have developed a wide array of antipredator behaviours to confront, dissuade or escape a predator. Death feigning (DF), where prey animals get in a tonic immobile state and resemble dead animal, is a sort of a last-ditch attempt to escape consumption. Even if this behaviour works, it is vitally important that the prey chooses the right time to escape: a quick decision may attract the predators' attention, while waiting too long might prolong exposure. In the field, we measured both the occurrence and the duration of DF in three distinct colour morphs of dice snakes; the most common dice morph, uniformly green, and melanistic individuals. We sampled only adult individuals and considered factors such as sex, size, body temperature, injuries, and presence of food, gravidity as well as absolute crawling speeds on the occurrence and duration of DF. Our results suggest that females DF more often than males and that gravid females DF the least often. Snakes that crawled faster were less likely to DF, but interestingly, gravid females that crawled faster were more likely to DF. Diced and green gravid snakes spent far less time in DF than nongravid individuals, while in melanistic snakes gravidity did not affect DF duration. These differences are especially pronounced in the uniformly green snakes. Additionally, colder, bigger and faster snakes DF for longer intervals. Larger snakes cannot be immediately consumed and thus can afford to try a risky strategy such as DF, which is supposedly used as a hard reset in a predator-prey interaction. Gravidity undoubtedly imposes significant constraints on snakes and probably limits escape options and makes DF too risky. However, melanism can offer certain advantages to gravid individuals, notably thermal benefits and in turn a higher metabolic rate and locomotor capacity which can prove beneficial in a DF situation.

Oral presentation

The consequences of interspecies relations between different species of rock lizards (*Darevskia*)

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Rock lizards of *Darevskia* genus is a well-known model for the study of evolution of multispecies systems which include parthenogenetic species. Although the hybrid origin of parthenogenetic species has been realized and supported it is still hard to understand which evolutionary forces driven the appearance of unisexual species and what evolutionary future they would have. Today, hybrids between parthenogenetic and parental species occur in the secondary overlap areas, where unisexual females also bear mating marks. The ratio of individuals belonging to parthenogenetic or bisexual species within such “mixed” populations can vary from almost absent members of gonochoristic species to almost equal number of females belonging to unisexual and bisexual species. For example, the proportion of adult *D. valentini* (bisexual species) to parthenogenetic *D. armeniaca* in Mets Sepasar population was 1:15 (total number of captured individuals was 125 during period 15-17 July 2021).

To reveal what is happening when parthenogenetic and bisexual species occur in syntopy, and how males choose between females of their own and parthenogenetic species, we observed their behaviour in several populations. We compared intersexual reactions of males and females in populations in syntopy with those from the monospecies populations. We found that social behaviour repertoire of parthenogenetic species is adequate in terms that their reactions don't differ from the reactions of females of bisexual species. Though male reaction on females of their own species and parthenogenetic species is more or less the same, it is more likely that they prefer to mate with the females of their own species. About 57% of adult parthenogenetic females had mating scars on their bellies. This fact might be crucial for survival of bisexual species within the areas of their coexistence with parthenogenetic relatives.

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Oral presentation

Sociability and aggressivity of insular Italian wall lizard vary in dependence on ecological conditions

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Behavioural traits can allow individuals swift adaptation to novel environmental conditions. Both sociability and aggressivity are known to vary greatly in dependence on population density. On small islands, where populations recurrently reach high densities, individuals usually decrease aggressivity and increase sociability to reduce needless energy expenditure and enhance their fitness. However, on islands where environmental conditions limit the population density, an elevated level of within-population aggressivity can be observed. To further test this phenomenon, we studied lizards in the Lastovo archipelago, where in 1971 Nevo and colleagues performed translocation of Italian wall lizard (*Podarcis siculus*) from the island of Pod Kopište to the island of Pod Mrčaru. Follow-up studies of that experiment showed that *P. siculus* populations from those two nearby islets differ in population density and in the number of phenotypic traits. As a part of the Genraliz project, which aimed to determine whether observed phenotypic differences were the result of genetic differences or phenotypic plasticity, we studied sociability and aggressivity of *P. siculus* populations from both islands. In the spring of 2017, we caught 46 adult lizards from the island of Pod Kopište and 40 adult lizards from the island of Pod Mrčaru and subjected them to behavioural testing after acclimatisation in captivity. Sociability test was performed in a 3-chambered sociability apparatus, while aggressivity was assessed by staging encounters in an open field apparatus and measuring aggressive displays. Preliminary results indicate that the population from Pod Mrčaru, which displays a higher population density in nature, has higher levels of sociability and lower levels of aggression, indicating behavioural adaptation to the new environmental condition.

Oral presentation

The effect of chemosensory enrichment on habituation in captive common wall lizards (*Podarcis muralis*)

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Environmental enrichment in captive environment increases animal wellbeing, reduces stress and abnormal behaviour. Research on environmental enrichment has mostly focused on birds and mammals, with chemical stimuli being employed rarely for enrichment. Chemical cues play an important role in reptilian biology as they use them to detect predators and prey as well as for social communication.

Podarics muralis is one of Europe's most widespread lizard. We believe any challenges faced by *P. muralis* - not only in captivity but also in isolation - are comparable to those encountered by other lizard species kept as laboratory animals for scientific purposes. Furthermore, behaviour and chemical communication of the genus *Podarcis* have been researched extensively, indicating *P. muralis* as a good experimental model. We conducted our research on 40 lizards caught in Zagreb, Croatia.

Lizards were kept in a controlled laboratory setting for two weeks and were exposed to lizard scents collected on filter paper from donor conspecifics of the opposite sex during both acclimatization and testing periods, whereas the control group received unscented filter papers. The open field test was used twice daily for 5 days to assess their behavior.

Using Ethovision XT15 software, we compared the rate of occurrence of different variables (i.e., tongue flicks, escape attempts, time spent in the centre of the open field, time spent moving, and distance travelled) to see if chemosensory enrichment affects lizard behaviour.

Our findings suggest that both groups become habituated during the experimental protocol. In enriched individuals, we found a sharp decline in assessed variables. This suggests that enriched lizards adapted to the exploration test faster, indicating that enrichment improves the ability to adapt to novelty. Given the importance of chemoreception in reptiles, chemosensory enrichment is a straightforward method that could possibly benefit a wide spectrum of captive species.

Oral presentation

Turtles racing for resources: Locomotor performance of sympatric populations of *Emys orbicularis* and *Mauremys rivulata*

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The rigid shell of turtles imposes significant locomotor constraints, especially in the terrestrial part of their habitat. Differences in mobility may affect the use of resources within their habitat: reaching favourable basking spots, food, mates and nesting places. We tested righting speed and efficiency of releasing from a rope, to compare mobility of sympatric populations of European pond turtle and Balkan pond turtle. When turtles were placed on their back, they spent some time inspecting their surroundings (TI), before they started overturning. Heavier individuals and those with a narrower carapace had shorter TI. We also found differences between the species and sexes, where the TI was the longest in *M. rivulata* females and shortest in *E. orbicularis* females. Although all turtles managed to right themselves promptly after the first righting attempt (max 44 s), *E. orbicularis* was significantly faster. Additionally, lighter individuals were faster to turn over when flipped on their back. Interestingly, the variability in turtle's locomotor abilities was only visible in the two parameters of righting performance. Releasing from the "vegetation alike" rope attached to the gular plates of the plastron was promptly resolved (max 48s) and we found no significant influence of species, sex or morphology. Our results imply a slight advantage of the European pond turtle when overturned, which might be an indicator of greater experience or morphological advantage for this particular task. This is probably due to a narrower carapace shape and a lighter body. The heavier body and the wider shell of Balkan pond turtles may provide some other advantages in locomotion such as greater stability and muscle strength. These characteristics would enable *M. rivulata* to move quicker toward favourable resource or push off the lighter and smaller *E. orbicularis*. Further studies are needed to test these hypotheses.

Oral presentation

Testing the effects of captive environment on individual variation in feeding behaviour of juvenile Hungarian meadow vipers (*Vipera ursinii rakosiensis*)

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The Hungarian meadow viper (*Vipera ursinii rakosiensis*) conservation program started captive breeding of the species in 2004. Over the past years altogether over 3800 vipers were born in the Hungarian Meadow Viper Conservation Centre and over 700 vipers were released to eight habitats in Kiskunság and Fertő-Hanság National Parks in Hungary. As captive environment potentially affects development of individual behavior and consequently, future survival of reintroduced vipers, we aimed to test effect of various environmental variables with the intention to fine-tune future captive breeding techniques. Using 32 juvenile vipers, representing 10 families, we assessed whether different prey items, origin and vipers' sex and structural diversity of captive environment affect individual feeding behavior. Vipers were kept in individual terraria and were offered crickets (*Acheta domestica* and *Gryllus assimilis*) every 4 days over a 6 week period. Feeding behavior was observed in a standardized way: we recorded individual timing of the first reaction and frequency of strike(s), holding and swallowing the prey item. According to first results, behavioral variables showed no or low repeatability indicating the lack of individual behavior strategies, which might root in the simple environment. Sexual difference was observed as males were generally faster in the first strike. We also detected feeding preference for house crickets over banana crickets, which is probably in line with substantial differences observed between prey species.

Oral presentation

What steers you to turn over? Analyses of European Pond Turtle righting success and performance

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The unique body plan of turtles is most notably recognized by the presence of the bony shell that covers most of its body. Although armored species are well protected against predation, that safety comes with the price of limitations in locomotion. When turned on their backs, turtles potentially risk being attacked by predators, or they can encounter problems such as hard breathing or overheating, which could end tragically for them. The ability of turtles to turn over as fast as possible can affect their fitness. The main question raised in this study is – what does righting success (RS) and performance (RP), depend on? Experiments addressing these questions were performed on the wild populations of the European Pond Turtle (*Emys orbicularis*) in their natural habitat, in four localities across Serbia. RP was measured as the time elapsed from the first attempt to turn over, until getting in the upright position. Turtles which failed to upright during three minutes period were considered unsuccessful (RS). Additional information about the turtle's morphology and body temperature was collected. Interestingly body temperature did not affect RS or RP. RS significantly differs among populations. RS is positively correlated with relative neck length, and negatively correlated with body mass. RP is significantly affected by the neck length, shell height (SH) and flatness index (FI). Results show that individuals with longer necks tend to turn over faster. Also, individuals with borderline maximal values for SH and FI, may be slower to turn over. As results suggest, large individuals, with relatively short necks, and flattened carapace may be slow to right, if they are able to right at all. These results could support the idea that RS and RP are attributes based on which natural selection could favor optimized neck length, body size, and shape of the shell.

Oral presentation

How good are European tree frog (*Hyla arborea*) tadpoles in hiding?

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Amphibian tadpoles can avoid threats through defensive coloration and defensive behavior. Another response to the exposure to threats is shape modification. We studied if and how quickly can *Hyla arborea* tadpoles achieve crypsis, by changing skin pigmentation, when introduced to environments with different background colorations. We also analyzed the reversibility of the skin color change and how different backgrounds affect tadpole body length, shape, and ontogenetic pathways. In addition, we tested if tadpoles can perceive their own coloration and actively choose an appropriate background. For this purpose, we reared tadpoles on white and black backgrounds. Half of the tadpoles from each treatment were switched to the alternative background halfway through the experiment. Coloration and length data were collected six times during the experiment, while data for shape and ontogenetic pathways analysis were collected twice (before the switch and at the end of the experiment). The behavioral test was performed at the end of the experiment. Our results suggest that *H. arborea* tadpoles can rapidly respond to the changes in the environmental background and this color change is reversible, but adaptation to the white background is not that good. Even though some physiological costs of rapid color change, especially in switched treatments, surely exist, according to our results this stress is not great enough to be expressed in variation of tadpoles' body length. However, some shape differences between treatments were evident. Tadpoles who were initially introduced in the white background had deeper tail fins and more pronounced snouts, a typical shape response when predation risk is perceived. Our results, also suggest that *H. arborea* tadpoles can recognize their own coloration and actively choose appropriate surroundings to maximize crypsis. This study represents the basis for the future analysis of adaptive coloration as it has a very complex function in anurans.

Oral presentation

Every snake for itself: Antipredator behavior of two syntopic populations of *Natrix* snakes

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Unlike other snake species, which commonly bite and vigorously try to escape predator's grip, the closely related semi aquatic grass snake (*Natrix natrix*) and the dice snake (*Natrix tessellata*), release cloacal secretions and display tonic immobility (TI) when threatened by a predator. TI is characterized by a relaxed body lacking any movement. This state can be accompanied by mouth opening when the animal is trying to simulate death i.e. death feigning (DF). The research was focused on a single locality where grass snakes and dice snakes occur syntopically, under similar conditions of predator pressure and available hideouts. Initially, we recorded behaviors that occurred half a minute after the capture. Grass snakes were significantly more likely to display DF than dice snakes when captured (21% vs. 2,6%, respectively). To assess the occurrence of tonic immobility we employed the same method for both species, i.e. 20 seconds of predator-simulating manipulation. The results indicate that there was no notable difference between species in the frequency of TI. Furthermore, the effect of sex and age on TI was not present in either species. Among grass snakes, adult individuals displayed more tendency toward TI when compared to juvenile ones (32.5% vs 29.6%, respectively).

Oral presentation

Sisterhood in reptiles: how and why females of viviparous lizard *Zootoca vivipara* aggregate together?

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Interactions between females are one of the blind-spots in our knowledge of reptilian social life. Apparently, females more often try to avoid same-sex individuals to decrease intrasexual competition. But sometimes we see deviant behaviour scenarios when females interplay and even cooperate together. So, studying the factors that encourage females to interact with each other is the key point in explaining this phenomenon. We studied *Zootoca vivipara* population in Tver Region (Russia) in years 2020 and 2022. Study area was a small meadow withing the taiga forest with a flock of rotten logs in the center. Most individuals displayed solitary social behaviour in spring, when females had small individual ranges (1–3 m²) that did not overlap with each other, and males overpassed huge areas (up to 50 m²), searching for females and their ranges overlapped broadly. In June several gravid females aggregated within permanent basking sites on the logs, uniting in small groups. The composition of their groups was constant and comprised from two to four individuals. After giving birth, the females left into the grass and no longer met each other. This bonding behaviour has been observed during the season 2020, but not in 2022, when we also observed gravid females. Interestingly, solitary lizards suddenly gather together and spend part of their time in groups with stable composition. Incentive for interaction may be associated with a specific phenological stage. Rattlesnakes *Crotalus horridus* is a rare example of female grouping based on kinship, whereas females aggregate together to increase effectiveness of eggs heating. We suggest, that cooperation of females of viviparous lizard in 2020 was due to relatively low ambient temperatures and might be also cooperation for joint incubation.

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Biology and evolution of behavior

Oral presentation

Consistency on the cold-hot types continuum: thermoregulation and personality study in viviparous lizard

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Given the strong relationship between thermoregulation and behavior in reptiles, as in ectotherms all life processes depend on achieving an optimal body temperature, thermoregulatory behaviour could potentially be structured into a syndrome. Thermoregulation in cold mountainous habitats is especially challenging, as it requires continuous thermoregulatory strategic decisions while animals also have to focusing daily tasks such as feeding, avoiding predators or even meeting seasonal goals like reproduction. According to our results from a common garden experiment, male common lizards (*Zootoca vivipara*) keep their body temperature in an accurate level (T_{sel} , V_{max}) and narrow range (T_{set}). On top of that, these thermal traits show consistent individual differences, therefore constitute an axis of animal personality, at least on a short term period. Locomotor activity in a familiar environment, risk-taking and shelter use show similar individual consistency. However, presence of behavioral syndrome (i.e. between-individual correlation) was only detectable across the individuals' thermoregulatory accuracy (T_{sel}) and risk-taking behavior: bold lizards being active on higher body temperatures. Furthermore, individuals show consistent differences in both environment-induced (behavioral plasticity) and environment-independent (behavioral predictability) within-individual behavioral variability.

Oral presentation

Factor in fear? Disentangling the roles of exploitative versus interference competition in disruptive selection

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Disruptive selection arises when extreme phenotypes have a greater fitness advantage compared to the intermediate phenotypes between them, and is regarded as an important source of variation in natural populations. Current theory suggest that by causing negative frequency dependent interactions, intraspecific resource competition is one of the key drivers of disruptive selection. However, intraspecific competition for resources can take different forms and be either indirect (exploitative competition) or direct (interference competition). To our knowledge, no previous study has investigated the role of interference competition in disruptive selection, and most general models of disruptive selection assume competition is exploitative. We experimentally investigated whether the type of competition is relevant in competitive interactions using a system where disruptive selection is common: the Mexican spadefoot toads (*Spea multiplicata*). Tadpoles of this species develop into one of two extreme phenotypes; carnivore morphs consume mainly fairy shrimp and other tadpoles, and omnivore morphs, with smaller jaw muscles and larger intestines, feed mainly on algae and detritus. Previous studies have shown that intermediate morphs generally have much lower fitness when competition is intense, as they are outcompeted by the specialized tadpoles for both resources. Our experiments revealed that (i) the presence of carnivores significantly modified the foraging behaviour of the focal (intermediate) tadpoles, and (ii) intermediate tadpoles exhibited significantly lower growth rates in direct (interference) competition treatments in comparison to exploitative competition treatments, when in competition with carnivores but not omnivores. These results suggest that interference competition may play a greater role than currently believed in driving disruptive selection. As carnivorous tadpoles are also cannibalistic, the 'fear' effect may have a greater impact on intermediate tadpoles than exploitative competition or consumption alone for their shared resource, similarly to how nonconsumptive effects can alter prey ecology and evolution more than consumptive effects in predator-prey or intraguild relations.

Oral presentation

Bite or run? Ecomorphological relationship in African agamas

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Understanding the relationships between form and function can help us to understand the evolution of phenotypic diversity in different ecological contexts. Many animals display morphological and behavioural adaptations to the habitats in which they live and the resources they exploit. Whole-organism performance traits such as bite force and locomotion are ecologically relevant as they reflect the ability of an organism to include a wider diversity, different or differently sized prey, to escape from predators, or to defend territories.

Here, we investigate ecomorphological relationships between diet, morphology, bite force and locomotor performance (sprint speed and endurance) in six species of South African agamids from three habitat types (ground-dwelling, rock-dwelling, and arboreal). Diet analyses showed that ants are a major food source, alongside with active prey such as beetles, wasps and flies. Body and head size are not directly related to diet, although greater in-levers for jaw closing (positively related to bite force) are associated to an increase of hard prey in the diet. Rock-dwelling species have a relatively flatter head than other species, possibly as an adaptation for crevice use. However, even when correcting for jaw length and jaw out-lever length, rock-dwelling species bite harder than ground-dwelling species. Further, endurance capacity of these lizards is correlated with hind limb and toe lengths. Interestingly, however, we demonstrate trade-offs in performance where specialisation towards speed comes at the detriment of endurance. Arboreal species have longer hind limbs, and higher exertion capacity and mean speed. However, for a given hind limb length, they appear to be slower than the other habitat specialists. In sum, our study illustrates the role of morphology in driving functional changes and how habitat use impacts morphology and locomotor performance but not bite force.

Oral presentation

Decrypting reptiles' behaviour through small size accelerometers

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Understanding animal behaviour is fundamental when studying a species biology, and an important precondition in wildlife ecology and conservation. The continuous technological advances have made available many variants of small animal-borne sensors able to effectively improve the study and analysis of animal behaviour. This paper, demonstrates how small animal borne tri-axial accelerometers can be used to record, identify, and comprehend the motion of small Mediterranean reptiles namely *Stellagama stellio* and *Dolichophis jugularis*. This is a first time that such an approach is followed for reptiles. A large set of motions have been recorded in a controlled lab environment (OptiTrack cameras at 100 fps) and in the field (RGB cameras at 30 fps). The recorded videos and acceleration curves were synchronized and studied as to identify and label selected motions (e.g. head bobbing, climbing, slithering, attacking). Using DDMT software, the acceleration characteristics of each labelled motion (including Pitch, Roll, Heading, and their differentials) were identified and parameterised allowing the automatic identification and marking of similar motions throughout a large dataset without the need of visual verification. This procedure provides insights on how the accelerometer data can be used to retrieve motion patterns and understand the behaviour of the reptiles in their habitats. The captured motions, behaviour patterns and their acceleration imprints have been developed into a Behavioural Pattern Database (BPD), while selected motions have been replicated in a digital environment using accurate 3D avatars of the studied animals. Even though this approach has been developed and tested on two Mediterranean reptiles it provides a detailed framework which can be replicated in other species with similar behaviour or body shape. Our approach can enhance research on animal behaviour by contributing to the analysis of complex or isolated behaviours, such as signals and social interactions, that are poorly studied.

Poster presentation

Barking Dogs Never Bite: Bluffing behaviour in dice snakes (*Natrix tessellata*)

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Prey animals have a wide array of strategies that they use to avoid or deter predators. In some cases, these strategies include warding off a predator attack by advertising strong chemical defences, such as venom, or by mimicking sympatric species, which do possess such defences (i.e. bluffing/mimicry). Dice snakes are nonvenomous natricine snakes that often live in sympatry with vipers. These snakes also have an ample arsenal of antipredator behavioural displays that include bluffing, characterized by hissing (auditory mimicry) with head flattening and fake strikes (visual mimicry). We measured the occurrence of bluffing displays in four different localities; on Golem Grad Island and in Konjsko (North Macedonia), where dice snakes share their habitat with nose-horned vipers (*Vipera ammodytes*) while in the other two localities, Mal Grad Island (Albania) and Pančevački rit (Serbia), vipers are not present. We analysed the effect of locality, size (snout to vent length) and sex on occurrence of bluffing as an antipredator strategy. Our results show that the occurrence of bluffing was significantly affected by locality and body size. Notably, in the two localities where dice snakes share the same habitat with vipers the occurrence of bluffing displays is significantly higher than in the two localities where vipers are not present. This possibly indicates a case of Batesian mimicry, reported for the first time in dice snakes. Additionally, our analysis shows that the predicted probabilities of the occurrence of bluffing increased with size. This seems intuitive, as bluffing behaviours should have a certain size threshold when they become effective, since small snakes can be easily consumed.

Poster presentation

Case of arboreal behaviour of common toads *Bufo bufo* in Poznań, Poland

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During an overnight field visit, we observed foraging and using arboreal ambush sites by common toads (*Bufo bufo*) and common frogs (*Rana temporaria*) in the protected area 'Traszkowski Ratajskie' within an urban park in Poznań. This area is covered with a scattered park stand, not exceeding 40% canopy cover, consisting of a mix of native and foreign species. On 20 June 2020, we detected 11 individuals of common toads (9 adults and two juveniles) and two common frogs located directly on the trees. The amphibians were mainly found on several white willow *Salix alba* and dead birch *Betula sp.* All these trees are old specimens with many trunks inclined at an angle, numerous cracks and partially decayed and covered with moss. We found no toads on other trees with similar characteristics despite a detailed check. The toads were found at the height of 40 to 220 cm (124.20 cm on average), while the frogs were at 30 to 40 cm. During the observation, toads were feeding on insects and slugs, which emerged en masse and despite our presence, they did not try to escape. We did not detect any other adult toads within the terrestrial habitats in the protected area, but we found a group of wild boars, *Sus scrofa* (several adults and almost 20 piglets), who escaped from the study area. As far as we know, this is the first detection of the mass arboreal behaviour in European common toads. Previous case studies described only single events of climbing toads in Denmark and toad presence in tree holes in the United Kingdom. We speculate that this behaviour is induced by external factors such as the risk of wild boar predation in combination with drought periods and the availability of shelters and prey on partial deadwood and mature trees.

Poster presentation

The behavior of amphibians during the winter months in the Orlja thermal springs, Olovo Municipality, Bosnia and Herzegovina

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The Orlja River belongs to the hydrographic system of the Krivaja River in Bosnia and Herzegovina and is unique in the fact that it is formed by two springs, one of which is thermal with a constant year-round temperature of 24.5°C. This area is, according to the main climatic characteristics located in the zone of temperate continental climate, with the average annual temperature being 10.0 °C. The coldest month is January with an average monthly temperature of -0.7 °C, and the warmest month of July with an average monthly temperature of 19.6 °C. Research on amphibians in the area of Orlja thermal springs near Olovo (Central Bosnia and Herzegovina) was conducted during the winter months (between October 2021 and March 2022). During the study period, we identified *Lissotriton vulgaris*, *Ichthyosaura alpestris*, and *Pelophylax ridibundus* populations that were active during the winter months. Due to the constant water temperature of thermal springs, amphibians of these three species remain active and do not hibernate. Besides the lack of hibernation, changes in the reproductive cycle were observed, as well as the appearance of gigantism in tadpoles. A similar observation has not been recorded in literature so far in Bosnia and Herzegovina.

Poster presentation

Hide or die when the winds bring wings: predator avoidance by activity shift in a mountain snake

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Basking time often increases the risk of predation and one way to minimise this risk is to reduce activity time and to stay within a refuge. However, this implies costs of lost opportunities for foraging, reproduction, and thermoregulation. We aimed to determine the main potential and actual predators of *Vipera graeca*, to estimate the incidence and the age and sex distribution of predation events based on body injuries to infer predation pressure, and to assess whether and how the activity of *V. graeca* individuals is modified by predation pressure. We surveyed 14 of the 17 known populations of *V. graeca*, examined 319 individuals for signs of predation-related injuries, and collected observational data on the daily activity of predators in the two largest *V. graeca* populations, used thermobiological measurements (T_{body} , T_o) to estimate the window of potential activity for *V. graeca* and compared the potential and observed activity of both *V. graeca* and observed predators. We found a relatively high proportion (12.5%) of injured *V. graeca* individuals, with more injuries on the posterior dorsal than on the anterior dorsal and caudal body parts. The incidence of predation-related injuries increased with estimated age, were more frequent on females than on males and they occurred earlier in age for females than for males. Finally, two results suggested that vipers may adjust their diurnal period of activity due to predation because (i) their daily activity was bimodal, probably to avoid the mid-day peak in raptor activity, and (ii) there was only moderate overlap with potential activity because the observed activity of vipers shifted earlier in the morning and later in the afternoon than could be expected based only on thermobiological conditions. These differences in activity patterns were consistent in two large populations on separate mountain ranges.

Session: Conservation and population genetics



Ichthyosaura alpestris

Conservation and population genetics

Oral presentation

The evolution of midwife toads (*Alytes*) revisited: a genomic approach

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The advent of genomic methods calls to revisit the evolutionary history of organisms for which robust phylogenies are still lacking, especially when dealing with species complexes that frequently hybridize. We used RAD-seq data covering the whole known diversity of midwife toads (genus *Alytes*) to infer their evolutionary history and address unresolved taxonomic issues. Based on thousands of markers, we recovered a robust phylogeny for the entire genus, including a comprehensive, well-resolved phylogeographic framework for the *A. obstetricans* complex. The results shed light on cyto-nuclear discordances caused by past hybridization, which have blurred the taxonomy of several taxa (*A. almogavarii*, *A. o. boscai*), and the extent of introgression between them. As part of our study, we also carefully explored the influence of different sampling schemes and bioinformatic thresholds on tree reconstruction, showing that under certain conditions, several slightly different, yet robust topologies can be retrieved from the genomic data. While showing the benefits of genomic approaches to readdress the diversity of European amphibians and settle on long-lasting taxonomic debates, we also encourage for proper consideration of parameter space and sampling design to reach sound conclusions.

Conservation and population genetics

Oral presentation

Patterns of genetic diversity in two endemic lizard populations of Cyprus

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Intra-island evolutionary processes are generally under-studied despite their importance for understanding lineage divergence, especially in isolated, oceanic islands. Cyprus is such an island laying within a biodiversity hotspot with a unique geological history and geomorphology that offers a great opportunity for the study of intra-island evolutionary and biogeographic dynamics. In this work, the genetic diversity patterns and population structure of two endemic lizards of Cyprus, namely Shreiber's Fringe-fingered lizard (*Acanthodactylus schreiberi schreiberi* Sindaco & Jeremčenko, 2008) and Troodos lizard (*Phoenicolacerta troodica* Werner, 1936) were investigated by means of gene analyses and genome-wide polymorphisms. Specimens sampled from different localities within Cyprus were sequenced for three mitochondrial (mtDNA) genes (16S, cytb and ND4). Moreover, approximately 9000 single nucleotide polymorphisms (SNPs) throughout the genome were identified through double digested restriction-site associated DNA sequencing (ddRADseq). Results from demographic and population analyses on mtDNA data identified only subtle genetic differentiation and structure for certain populations. Phylogenetic and population structure analyses on ddRADseq data indicated that both Cypriot taxa are monophyletic and highly structured, with the resulting patterns reflecting distinct geographic entities within the island. When the two species were compared, their genetic diversity patterns show similarities indicative of the effect of Cyprus' palaeogeographic history and topology, providing new insights regarding the evolutionary and biogeographic processes at a local scale.

Oral presentation

Using genomics to reveal the diversity and biogeography of the Hajar Mountains' endemic reptiles

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The Hajar Mountains comprise a rather small, isolated mountain range in the southeast of the Arabian Peninsula. This mountain range is divided into three mountain blocks with a complex but well-studied orogeny and is considered a hotspot of reptile diversity in Arabia. They contain around 60 species of reptiles, 19 of which are endemic. All of the above make this mountain range the perfect location to study in-depth how reptile communities have colonized, diversified, and dispersed in mountain environments. In this study, we use genomic tools based on ddRAD sequencing to untangle the extreme diversity of the endemic reptile fauna of the Hajar Mountains and integrate biogeographic analyses at the species, lineage, and individual level to follow the evolutionary history of these species from their first colonization to contemporaneous dispersion events. We found support for up to 49 independent endemic lineages with two main mountain radiations in the geckos of the genus *Asaccus* and in *Pristurus rupestris*. Biogeographic reconstructions recovered 11 independent colonization events and we were able to locate the region of entry to the mountains of nine of them. We found rather low dispersion between the three mountain blocks, suggesting that they act as independent evolutionary units. Moreover, we found that vicariance events between blocks have increased more than would be expected by random diversification, especially in the last 5 My. This coincides with an ongoing aridification of Arabia, which may be forcing the endemic lineages to disperse towards higher altitudes, thus isolating them even more, and reducing their distributional ranges.

Oral presentation

Reliability of de novo assembly of ddRAD sequences for population genetics in non-model species

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Genotyping-by-sequencing (GBS), such as double-digest restriction site-associated DNA sequencing (ddRADseq), has the potential to be broadly applicable for studying population genetics at genome-wide single nucleotide polymorphisms (SNPs). The analysis of ddRADseq data requires merging DNA sequences into homologous loci, which can be easily achieved when a reference genome is available. For species lacking a reference genome, de novo SNP discovery and genotyping is the only alternative approach. However, this approach is challenging, as it requires a careful choice of parameters for reconstructing homologous loci. Here, we examined the genetic variability of three populations of the lizard *Podarcis siculus* using a de novo assembly, and explored the potential of this approach to generate reliable genetic data by comparing the results to a reference-based assembly using the genome of *Podarcis muralis*. We also evaluated the impact of sequencing length (80bp vs. 120bp), and thus of the number of SNPs, on the results. We obtained a similar number of loci using de novo (2,533) and reference-based (2,654) assemblies. The number of independent polymorphic SNPs was similar whatever the method and sequencing length (80bp: de novo: 378, reference-based: 377; 120bp: de novo: 428, reference-based: 437). We estimated genetic diversity indices and performed population structure analyses separately with the four different sets of SNPs, and found nearly identical results. Population genetics analyses all revealed weak genetic differentiation and one main genetic cluster, and the same variation in expected heterozygosity among populations. Our results support the idea that optimized bioinformatics pipelines and rigorous filtering provide a high-quality de novo reconstruction of homologous ddRADseq loci, and that the resulting genetic data are reliable for studying the genetic variability of populations.

Conservation and population genetics

Oral presentation

Genomic phylogeography as a lie detector to traditional molecular taxonomy in amphibians

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Amphibians feature both the highest rates of new species discoveries and species declines among vertebrates. To characterize and describe this diversity faster than it disappears, genetic analyses are strong allies of integrative taxonomy. In the toolbox of taxonomists, sequences of a few mitochondrial- and nuclear-encoding genes, combined with analyses of neutral, fast-evolving microsatellite markers, have been preferred for phylogenetic and phylogeographic analyses, especially in complex species groups where lineages lack clear phenotypic differences. The numerous limitations of single gene inferences, especially from mitochondrial sequences, has been known for decades, yet the validity and new descriptions of many amphibian species and subspecies still rely on such molecular evidence. During this talk, I will develop on recent published and unpublished work where we re-assessed the phylogeography of several species complexes of Palearctic anurans with genomic data obtained by RAD-sequencing, focusing on the discrepancies with conventional genetic markers. Instances of deep discordances are found in nearly all groups, including incomplete lineage sorting at universally used intron sequences that mimic conspecificity, high ancestral polymorphism at microsatellites that confound with admixture, or past and present mitochondrial introgression that alternatively hides or give false impression of cryptic species, all that have led or could lead to taxonomic confusions. The prevalence of these hidden patterns in the supposedly well-known herpetofauna of the Western Palearctic predicts numerous errors regarding the distribution, nomenclature and taxonomy in lesser studied regions of the world. However, the potential unreliability of conventional markers must obviously not refrain the description of biodiversity when it is urgently needed. I will draw lessons from the genomic studies to propose some guidelines to cope with these limitations, illustrated by a recent overview of anuran diversity in Central and East Asia, where taxonomic changes based on little molecular data have skyrocketed in recent years.

Conservation and population genetics

Oral presentation

The intraspecific mtDNA differentiation of *Bufo verrucosissimus* (Pallas, 1814)

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The Caucasian toad, *Bufo verrucosissimus*, occurs throughout Caucasia, at north-eastern and south of Turkey as well as Lebanon. The species has been morphologically delimited under four different subspecies distributing in Caucasia, but recent genetic studies have indicated the presence of three different genetic lineages rather than morphological units. Besides, they have indicated the possibility of different genetic units along the southern distribution of the species. In this study, we have assessed the genetic diversity of *B. verrucosissimus* based on mitochondrial markers. For that, we investigated the intraspecific variation using the data belonging to 16S rRNA and Cytb gene regions obtained from GenBank. We constructed phylogenetic background for each gene and concatenated data, respectively. Regarding constructed phylogenies, we have determined the presence of five well supported lineages corresponding to the north of Caucasia, south of Caucasia including north-eastern Turkey, western and eastern Mediterranean regions at the south of Turkey and Lebanon. Moreover, the intraspecific assessment yielded more resolved relationships between lineages compared to the previous studies conducted on *Bufo bufo* species group including *B. verrucosissimus* data. Caucasia, Anatolia, and Levant, in which the species has characterized with different genetic units, harboured amphibian and reptile species as refugia during the Quaternary climate oscillations. These regions are representing deep genetic variation for numerous taxa shaped by allopatric isolation between populations due to tectonic events and subsequent glacial cycles. We also suggest that the observed mtDNA differentiation of *B. verrucosissimus* is relevant to the phylogeographic and cladogenetic histories along distribution range. Therefore, further studies can focus on intraspecific genetic diversity based on microsatellite markers combining with morphology to handle subspecies situations.

Oral presentation

The Alpine clade of *Vipera berus* – a new but still puzzling taxon for the European snake fauna

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In 2004, the late Svetlana Kalyabina and co-authors discovered a clade of Alpine adders (*Vipera berus* complex) which were genetically different from *V. b. berus* and *V. b. bosniensis*. This „alpine clade” was subsequently identified from Switzerland, Austria, extreme SE Germany, northern Italy and Slovenia. Its glacial refugia were probably south of the Alps. In 2020, Schmidler and Hansbauer gave it the subspecies name *V. berus marasso* (Pollini, 1818). This taxon had been described from a lowland population in the Po valley, northern Italy. This population is now extinct, therefore its identity with the Alpine clade is difficult to check. Morphologically, the Alpine clade has not yet been characterized. A diagnosis is pending.

We checked mitochondrial sequences and microsatellites in three populations in the contact zone of ‚*marasso*’ with *V. b. berus* in SE Bavaria. We discovered discrepancies between mitochondrial and nuclear gene status which indicate a possible male mediated gene flow via migration in East-West direction. Population studies in Austria and northern Italy/Slovenia, as well as sequencing of museum specimens are needed to clarify the nomenclatorial status of *V. b. marasso*.

Oral presentation

Lack of reproductive isolation between the two subspecies of the European whip snake *Hierophis viridiflavus*

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The two subspecies of the European green whip snake *Hierophis viridiflavus* *viridiflavus* and *H. v. carbonarius* represent an interesting model system to study speciation. Indeed, they likely evolved isolated during glacial periods due to isolation by distance even if the species is considered as highly mobile. Recent studies show that even though these subspecies belong to different mitochondrial lineages, a single tested nuclear gene and morphological traits display overlapping character states. Moreover, little is known whether these two subspecies are reproductively isolated from each other. Furthermore, it is unknown how the complete or partial melanism of the subspecies *carbonarius* is regulated and why it is restricted to this eastern subspecies only. Therefore, we evaluated the amount of gene flow within two transects across the contact zone of the subspecies with a genomic approach using double-digest restriction site-associated DNA (dd-RAD). Our molecular samples, including 148 individuals from the contact zones and further away, were genotyped on 24,817 single nucleotide polymorphisms (SNP). Admixture analysis supports the existence of two well-defined clades corresponding to both subspecies. However, a high gene flow between both subspecies was observed within a cline of 300 km. Moreover, associations between phenotypic characters and genomic markers were investigated by quantifying the amount of yellow coloration and geometric morphometrics were conducted to determine morphological differentiation between both subspecies for a sample of 40 genetically admixed individuals. We conclude that the two subspecies represent evolutionary significant units (ESU). Due to the amount of gene flow in a very large area, it seems that post-zygotic reproductive barriers have not evolved yet. On a taxonomic basis, we suggest continuing treating the two ESU as subspecies.

Oral presentation

Conservation genomics shows limited negative effects of isolation in an endangered venomous snake living on sky islands

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The meadow-steppe viper complex (*Vipera ursinii* complex) includes some of the most endangered vertebrate taxa in Europe. The threatened *Vipera graeca* is sister to all other clades of *ursinii* and *renardi* in the *V. ursinii-renardi* complex and thus forms a basal lineage. Our study aimed to explore phylogenomic and conservation genomic characteristics of *V. graeca* by addressing (i) phylogenomic relationships between populations, (ii) refugia during the last glacial maximum, (iii) current gene flow between populations, and (iv) evidence of inbreeding within populations. A RADseq library was prepared from samples from all known populations (n=18) and sequenced using the Illumina NextSeq 550 platform. Polymorphic positions of RAD loci were identified by aligning the sequencing reads to the draft genome of *Vipera berus*. The phylogenetic structuring of *V. graeca* populations showed a strong latitudinal pattern: the central and southern populations were more phylogenetically structured, and more northerly populations showed little differentiation. Structure analysis did not identify distinct clusters in the northern populations. Nei’s genetic distance correlated significantly with geographical distance, thus, an isolation-by-distance pattern was inferred. Small populations had lower observed and expected heterozygosity, but their inbreeding coefficient showed no significant deviance from zero, suggesting no inbreeding in the studied *V. graeca* populations. Southern populations showed pronounced genetic differentiation, while the northern ones displayed little structure corresponding to a classical “rear edge–leading edge” phylogeographic structure that was presumably formed by several extinction-colonization events during the Pleistocene. Despite the lack of genetic differentiation between the northern populations, there is probably no ongoing gene-flow between these populations.

Conservation and population genetics

Oral presentation

From trees to *Triturus* – why we need to rethink our approach to protected areas for amphibians and reptiles

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We live in a time of biodiversity crisis, and amphibians have been hit more severely than any other vertebrate class. Reptiles have fared little better. Traditional approaches to conservation have focussed on species or habitats, but have neglected the third element of biodiversity; genetics. However, a recent review of the effectiveness of protected areas suggests that they have not been effective enough to halt biodiversity loss. We propose taking an approach from the sphere of tree conservation, the Gene Conservation Unit (GCU). GCUs aim to allow dynamic processes to take place, thereby fostering adaptation. Habitat loss, particularly through urban expansion lead to population loss and concomitant diversity loss, as well as isolation. Herps are particularly susceptible to loss of genetic diversity due to their relatively limited powers of dispersal and this may be reflected in loss of fitness through inbreeding depression and increased susceptibility to parasites and pathogens.

We present two case studies of in situ conservation of genetic diversity in amphibians: one planned, the other a by-product of climate change mitigation. These show that GCUs can be a viable approach. A survey of UK land managers and conservationists also showed high levels (90%) of support for fully integrating genetic information into conservation management. We believe that by fully incorporating genetic diversity in the design of protected areas will make them more resilient and better able to support amphibians and reptiles through these times of rapid change.

Conservation and population genetics

Oral presentation

Conquering taxonomic impediments within cophyline frogs (Anura: Microhylidae: Cophylinae) using a type-specimen explicit protocol for integrative species delimitation

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DNA barcoding studies of Madagascan frogs revealed a considerable undescribed diversity within the microhylid subfamily Cophylinae, a clade endemic to Madagascar with currently 115 described species subdivided in nine genera. Although taxonomic progress over the past years almost doubled the number of recognized species within the group, about 50 species are still awaiting taxonomic description. The description of some of those so-called candidate species, however, is hampered by unclarified taxonomic names, and unresolved species complexes. Type specimens are thus a valuable source of information, as they allow for the unambiguous allocation of taxonomic names. But sequencing genetic markers from archival samples oftentimes proves to be challenging due to degradation of DNA over time or the preservation techniques used. Our project aims to overcome these taxonomic impediments by using a state-of-the-art hybrid-enrichment sequencing method called FrogCap, targeting about 13,000 nuclear markers, which will be applied to fresh material of all available species. Subsequently, a newly developed bait set derived from FrogCap, called MuseoFrogCap, will then be used on type material. Preliminary low-coverage shotgun sequencing has shown that extractions were successful from at least 51/74 museomic specimens, and already suffices for some preliminary conclusions. Once hybrid-enrichment sequences are available from modern and museomic material, we will be able to integrate them in phylogenomic analyses in order to tackle the species complexes in question, reassess the number of candidate species and provide an up to date phylogeny for cophyline frogs. This is the first part of our newly established type-specimen explicit protocol for integrative species delimitation, which, in a second part, will be complemented by the analysis of morphological and micro-Computed Tomography (micro-CT) data gathered from modern and historical specimens. This approach will not only allow us to resolve cophyline taxonomy once and for all, but also pave the way for macroevolutionary studies.

Oral presentation

Multiple independent transitions to cave life and high diversity in the olm

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The olm (*Proteus anguinus*) is probably the most exceptional amphibian in Europe. It occupies a unique phylogenetic position, with its closest relative being the Northern American mudpuppies (*Necturus*). Its habitat is limited to the subterranean waters of the Dinaric karst, with a seemingly uniform phenotypic appearance of cave adapted traits: an elongate body, snout and limbs, degenerated eyes and loss of pigmentation ('white olm'). Only a single small region in Southeastern Slovenia harbors olms with a phenotype more typical of surface animals: pigmented skin, presence of eyes and a blunt snout with short limbs ('black olm'). Yet, despite of its captivating and unique appearance, until now little was known on its genetic variability and evolutionary history. Here, we use a combination of intensive sampling with mitochondrial DNA and genome-wide SNP data to investigate the molecular diversity, evolutionary history and biogeography of olms along the Dinaric Karst. We find extraordinary diversity, with nine deeply divergent lineages that separated between 4 – 17 mya, while molecular diversity within lineages was extremely low. These show no signal of recent admixture and very limited amount of historical gene flow. Species delimitations show that these lineages can be considered separate species. The depigmented lineages also show clear differences between them, for example in head shape and limb size. Biogeographically, the contemporaneous distribution of lineages mirrors hydrologically separated subterranean environments, while the historical separation of olm lineages follows micro-tectonic and climatic changes in the area. The reconstructed phylogeny suggests at least four independent transitions to the cave phenotype, or alternatively a single transition and one reversal to the surface phenotype. Some lineages exhibit extremely small ranges, and coupled with the deep divergence between and low divergence within olms, this highlights the importance of protecting each lineage separately.

Oral presentation

Genomics reveal mitonuclear discordance and introgression in the desert-adapted vipers of the genus *Cerastes*

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The desert horned vipers (genus *Cerastes*) are a small monophyletic group of venomous snakes within the Family Viperidae. According to published morphological and molecular studies, the group is comprised by four species: two phylogenetically sister taxa, *Cerastes cerastes* and *Cerastes gasperettii*, with generalist habitat requirements and allopatrically distributed across the Saharan and Arabian deserts; a more distantly related species, *Cerastes vipera*, inhabiting the sandy environments of the Sahara desert, and the enigmatic *Cerastes boehmei*, only known from a single specimen in captivity allegedly captured in Central Tunisia in 1991. In this study, we analysed one mitochondrial marker (COI) and genome-wide data (ddRAD sequencing) from 101 and 24 samples, respectively, covering the entire distribution range of the genus to explore the phylogenomic relationships, population genomics, and introgression patterns within the genus *Cerastes*. In addition, and to provide insights into the mode of diversification of the group, we carried out niche overlap analyses considering climatic and habitat variables. Our results using more than 500.000 base pairs show an unexpected phylogenomic relationship in clear discordance with the inferences derived from the published mitochondrial and multilocus approaches, with *C. cerastes* and *C. vipera* as sister taxa and with *C. gasperettii* sister taxon to these two species. These results are also supported by the population genomic analyses using more than 2,000 unlinked SNPs. Niche overlap analyses show a relatively high niche overlap (OI > 0.6) both in climatic and habitat variables between *C. cerastes* and *C. vipera*, thus, contradicting a potential scenario of sympatric speciation. These results are in line with the ancient introgression found within the western populations of *C. cerastes* and *C. vipera*. Finally, our genomic data confirms the existence of a lineage within *C. cerastes* in Arabia, representing the subspecies *C. cerastes hoofieni*. All these results highlight the importance of genome-wide data over few genetic markers to study the evolutionary history of non-model species.

Oral presentation

So close, no matter how far': A remarkable new record extends the distribution, but not the genetic diversity of the Moldavian meadow viper (*Vipera ursinii moldavica*)

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Reptiles are amongst the vertebrates most prone to extinction, and this is true for many snake species, for which decades of continuous demographic decline have already been documented. In Central and Eastern Europe, species linked to lowland habitats such as grasslands are particularly threatened, as these have been overwhelmingly transformed into agricultural fields. One such species is the meadow viper (*Vipera ursinii*). Although the species is only classified as Vulnerable (VU) in the IUCN Red List due to a relatively stable situation of its montane populations from the Alps and Western Balkans, its two lowland subspecies (*V. u. rakosiensis* and *V. u. moldavica*) are endangered (EN) and critically endangered (CR), and are believed to have already become extinct from several countries. The easternmost subspecies of the *Vipera ursinii* complex, the Moldavian meadow viper (*V. u. moldavica*) is also the most threatened. Although, historically, it was recorded from Bulgaria, the Republic of Moldova, and five counties in eastern Romania, for many decades it was only known to have persisted in two counties from Romania: Iași, in the north-east, and Tulcea (the Danube Delta), in the south-east of the country. Here we improve the current knowledge of the distribution and conservation status of the Moldavian meadow viper in Romania. We update the species' distribution both within the recently confirmed regions for the species, present a remarkable new occurrence record from the Carpathian Bend area (Buzău county), and utilize species distribution modeling to identify new potentially suitable areas for the species. Finally, we sequenced mitochondrial and nuclear DNA markers from all currently known regions of occurrence, and ran phylogenetic analyses together with previously published sequences. Surprisingly, a complete lack of genetic variation was found for *V. u. moldavica*, suggesting a very recent dispersal and/ or fragmentation of a once extensive population.

Oral presentation

Extended insights into genetic diversity of alpine newts in the Julian Alps (Slovenia)

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The alpine newt *Ichthyosaura alpestris* is widely distributed in Europe. Populations in the Mediterranean peninsulas (Iberian Peninsula, Apennines, Balkans) harbour several subspecies and varieties that have been described mainly on the basis of external morphology. mtDNA and nuDNA analyses revealed considerable intraspecific differentiation with clades matching some of the proposed subspecies. Unfortunately, Slovenia was mostly omitted from comprehensive phylogeographic studies, although the Julian Alps harbour two distinct subspecies, *I. alpestris lacustris* and *I. alpestris lacusnigri*. The first subspecies, a predominantly paedomorphic population inhabiting a single lake, became extinct due to the introduction of fish, while the second subspecies still occurs in smaller numbers alongside typical alpine newts in a single fish-infested alpine lake. A previous study conducted in Slovenia suggests that alpine newts from the Julian Alps are genetically indistinguishable from surrounding populations and nested within the mitochondrial C3 clade, but these analyses were performed on a limited number of samples and used a single mtDNA marker. To gain a better insight into the genetic diversity of newts from the Julian Alps, we extensively sampled this region and other parts of Slovenia. We sequenced two mitochondrial (CytB, ND2) and one nuclear fragment (GH) from 185 individuals. According to the mtDNA they all belong to the western C3 clade, but this is only partially consistent with the nuclear data, as we found discordances between nuclear and mitochondrial markers. Between 5 and 10% of individuals from the Julian Alps, but not from other Slovenian populations, belonged to the eastern rather than the western lineage according to GH. These results suggest that populations from the Julian Alps are not only morphologically but possibly also genetically distinct from other Slovenian populations and require stricter protection and separate conservation measures.

Conservation and population genetics

Oral presentation

Genomic insights into a critically-endangered glacial relict – the Montseny brook newt

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The shifting climate of Europe during the Quaternary, besides a complex geography, has left behind copious examples of vicariance and disjunct ranges across the Old Continent. One of those relicts – the Montseny brook newt *Calotriton arnoldi*– diverged from its relatively widespread sister species, the Pyrenean brook newt *C. asper*, almost 2 million years ago. From then on, it has evolved in isolation at a Eurosiberian exclave, the Montseny Massif (Catalonia, NE Spain), where currently inhabits only eight mountain brooks. Such a tiny range, together with small population sizes, the outbreak of lethal emergent diseases, the rampant water extraction at the massif, and the threat of global warming, make this species the most endangered amphibian in Western Europe. Genomic data, obtained from 153 ddRADseq samples and summed up in 9,470 unlinked high-quality SNPs, shed light on the hierarchical structure of their populations, highly-fragmented by both human-related causes as well as biological constraints, which have left their signals along a huge but heavily-impooverished genome. Those genomic signals have allowed us to infer genetic diversity, delve into a demographic history as turbulent as the past European climate or reconstruct present and ancient migrations. Necessary to unravel their evolutionary history and evaluate the health of their populations, genomics raises as the new cornerstone of 21st-century conservation efforts, and coupled with ecological, geographical, and morphological evidence give us the opportunity to reassess the systematics from a truly integrated framework, a key step for the orchestration of any state-of-the-art breeding program.

Conservation and population genetics

Oral presentation

Systematics and biogeography of the colubrid genus *Platyceps*

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The colubrids genus *Platyceps* has been included in several phylogenetic studies of the family Colubridae, however the phylogenetic relationships between its species still remain unresolved. We analysed 90 specimens of fourteen *Platyceps* species, some of them have never been included in a genetic analysis before. The phylogeny is based on a combination of four mitochondrial (12S rRNA, cytb, COI, ND4) and two nuclear (cmtos, NT3) markers. Our results confirm the genus as monophyletic. Two species from India (*P. plinii*, *P. josephi*) form a separate clade. The rest is divided in three clades – Indian (*P. bholanathi*, *P. gracilis*, *P. ladacensis*, *P. ventromaculatus* and one undescribed species), West Asian (*P. karelini*, *P. rogersi*, *P. saharicus* and *P. rhodorachis*) and a widespread clade (*P. florulentus*, *P. taylori*, *P. najadum*, *P. collaris*, *P. elegantissimus*, *P. manseri*, *P. sinai*, *P. variabilis*). According to the results, the phylogenetic positions of several species differ considerably compared to previously published studies - *P. thomasi* appears to be a color variation of *P. variabilis*, *P. variabilis manseri* emerges as a distinct species, *P. insulanus* is genetically identical to *P. rhodorachis*, populations of *P. rhodorachis* in northern Iran, Turkmenistan, Uzbekistan, Kyrgyzstan and Afghanistan seems to be a separate species. The age of the *Platyceps* crown divergence is estimated to 19 million years ago, but the geographical origin of the genus could not be determined. Species from the West Asian clade originated in Asia from where they dispersed through the Arabian Peninsula to Africa. The origin of the species *P. najadum*, *P. collaris*, *P. elegantissimus*, *P. manseri*, *P. sinai* and *P. variabilis* lies in the Levant, from where they spread to Europe in the west and the Arabian Peninsula in the southeast.

Conservation and population genetics

Poster presentation

Patterns of diversity in three Ecuadorian amphibian and reptile species

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Ecuador exhibits some of the highest biodiversity proportional to land cover anywhere in the world. The Tropical Andes Biodiversity Hotspot running through central Ecuador is the most diverse Biodiversity Hotspot globally. Elevation and geographic barriers are proven drivers of genetic variation within species, leading to distinct lineages and speciation. Due to the complex geology in northwest Ecuador, highly variable habitats, and high biodiversity, further research is required to fill phylogeographic knowledge gaps in Ecuador. We investigate phylogeographic patterns from mitochondrial and nuclear sequence data generated for two frog (*Espadarana prosoblepon*, *Pristimantis achatinus*) and one lizard species (*Gonatodes caudiscutatus*). We will present maximum likelihood and Bayesian inference phylogenies as well as haplotype networks to assess the degree of genetic divergence within each studied species. We investigate the impacts that elevation, habitat type, geology, and anthropogenic disturbance on lineage formation or barriers to geneflow and identify areas for future work to focus on. In addition, we will present data on the likely origins of *G. caudiscutatus* on the Galapagos Islands.

Conservation and population genetics

Poster presentation

Conservation genetics of the Macedonian crested newt, *Triturus macedonicus* (Amphibia, Salamandridae), in Northern Pindos National Park

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In this study we used genetic approaches to assess the conservation status of the Macedonian crested newt, *Triturus macedonicus*, in Northern Pindos National Park (Epirus, Greece). Mitochondrial DNA sequences and multilocus genotypes from 38 breeding populations, were used to infer their phylogenetic position, as well as to detect and measure genetic variation patterns, population genetic structure and levels of gene flow. Examined populations fall within two major clades of the Macedonian crested newt phylogeny, being geographically separated by River Aoos' valley and Vikos' gorge. Both population groups constitute separate gene pools bearing private haplotypes and alleles and found to be highly differentiated in both mitochondrial (p-distance = 2.8%) and microsatellite markers ($F_{st} = 0.184$). Thus, they meet all the criteria to be characterized as Significant Evolutionary Units (ESUs) that deserve separate conservation status. Within each ESU, the particularly high inter-population differentiation and low migration rates imply lack of connectivity between breeding sites and local isolation in favourable habitats. Our results can inform future programs and actions, towards the conservation and management of the Macedonian crested newt in the area.

Poster presentation

New dwarfs from Madagascar: Molecular and morphological data reveal unseen diversity in Malagasy geckos, genus *Lygodactylus*

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The genus *Lygodactylus* comprises 71 valid species of small, diurnal geckos distributed across Madagascar, sub-Saharan Africa, and South America. A recently published multigene phylogeny inferred from ~10.1 kbp concatenated sequences by including 56 species of all 14 known species groups revealed four major clades and the existence of 34 candidate species, 19 of which are newly identified. This uncovered diversity is especially high in two Malagasy species groups: first, the mainly rainforest dwelling *L. madagascariensis* species group (subgenus *Domerguella*) from the northern and eastern parts of Madagascar, with 12 deep genetic lineages in addition to only five currently valid species; and second, the rather dry-forest and partly montane dwelling *L. verticillatus* group of the South and West of Madagascar which includes the genetically highly distinct *L. tolampyae* clade with at least six different undiscovered lineages. Genetic distances between all these lineages are exceptionally high, and several of them occur in syntopy without genetic admixture, leaving no doubt about their status as distinct species, even under a conservative biological species criterion. Research on the *L. madagascariensis* group will lead to the elevation of *L. petteri* to species level and the description of eight new species. Current molecular work on the *L. tolampyae* clade illuminates a comparably underestimated diversity and will lead to the description of even more species in this highly cryptic group of geckos.

Conservation and population genetics

Poster presentation

Phylogeographic patterns of the common frog (*Rana temporaria*) in the UK

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Genetic approaches are increasingly used to address questions in evolutionary ecology and conservation. There are limited published genetic data on common frogs, *Rana temporaria*, in the UK and a full appreciation of the genetic variation is unknown. Previous mitochondrial data of *R. temporaria* has identified little genetic variation between populations across England and Wales, with Scottish populations having multiple haplotypes. However, these previous works contained sampling at a limited number of UK sites. In this presentation we extend previous work and assess the genetic variation of *R. temporaria* across England, Scotland and Wales. Buccal swabs were collected from wild populations of *R. temporaria* from across the UK, including using a citizen science-based approach to increase our sampling area. Following successful DNA extraction from the buccal swabs, sequence data was generated for mitochondrial (cytb) and nuclear DNA (rag1) from ca. 40 localities. We present results from maximum likelihood and Bayesian inference phylogenetic methods, and haplotype networks to better identify genetic diversity across the UK.

Conservation and population genetics

Poster presentation

Population genomic survey of the Rio Grande cooter (*Pseudemys gorzugi*)

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Turtles are an integral component of freshwater ecosystems. They can obtain some of the largest biomasses among all vertebrate groups, assume roles in every trophic position, and serve as bioindicators of environmental quality. Among the least studied freshwater turtles in the United States of America (US), is the Rio Grande River cooter (*Pseudemys gorzugi*). The species is native to the lower Rio Grande Basin and its tributaries. Because of its limited distribution, we wanted to conduct a population genetic analysis of the species across its range in the US, to assess the genetic health of the species and identify populations of most concern (i.e., isolated populations with low genetic diversity). To do so, we isolated DNA from 141 samples collected throughout their range and performed double digest restriction-site associated DNA (ddRAD) sequencing to identify genome-wide single nucleotide polymorphisms (SNPs). We identified and assessed ~30,000 SNPs. Overall, STRUCTURE and PCA analyses suggested there were two genetically distinct populations, the Rio Grande population and the Pecos River population. F_{ST} metrics among populations was approximately 0.2, consistent with moderate-to-high levels of differentiation. Private alleles were most common in the Rio Grande and heterozygosity was higher in the Rio Grande. These results in combination with previous surveys suggest populations along the Pecos River are at most risk, (i.e., these populations isolated from the “source” population). This study demonstrates the importance of understanding genetic diversity of imperiled taxa and can aid in future conservation efforts, which should focus on restoring connectivity of the Pecos River.

Poster presentation

Phylogeographic substructuring of the common frog (*Rana temporaria*) in Serbia

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The common frog (*Rana temporaria* Linnaeus, 1758) is one of the most widespread and abundant amphibians in Europe, except in the southern parts of the continent, where distribution is apparently fragmented. It has the greatest genetic variability of all western Palearctic brown frogs and it is extremely variable in morphology and ecological preferences, also. The Republic of Serbia is a country of particular interest in terms of amphibian conservation because of the genetically and morphologically diverse populations of several amphibian species.

We analysed nucleotide variability of mitochondrial DNA sequences of partial MT-CYTB gene (461 bp) of 27 specimens of *R. temporaria* species. Samples (eggs and tail tips of adult individuals) were collected in two periods, 1986-2007 and 2013-2017, in 14 localities in Serbia. The analyses revealed nine different haplotypes separated into two main clusters: north-eastern and eastern (localities Bela Crkva, Grza, Bigar, Đerdap, Stara planina) and central, west, south-western, and south-eastern Serbia (localities Jagodnja, Lučani, Zlatibor, Goč, Kopaonik, Prokletije, Šar planina, Oštrozub, and Vlasina). Lučani and Grza populations had two different haplotypes each.

Our results confirmed previous findings that the common frog shows phylogeographic substructuring. The existence of two genetically diverged population groups on the territory of Serbia suggests that these populations should be adequately protected in terms of the conservation of their breeding places and suitable terrestrial habitats. In Serbia, *R. temporaria* habitats are currently not only fragmented but also under intensive anthropogenic pressure, due to intensive urbanization, particularly in the mountain areas and the promotion of non-sustainable tourism, which makes them highly vulnerable. Therefore, there is a need for more precise data about species' phylogeny and distribution, both at the national and regional level, to define conservation priorities in the near future.

Poster presentation

A comparison of approaches for DNA recovery of buccal swabs in a widespread Eurasian amphibian, the common toad (*Bufo bufo*)

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Buccal (oral) swabs are a reliable, minimally invasive method to collect DNA for PCR-based downstream analyses in many amphibian taxa. Buccal swabbing gained popularity as an important method of DNA collection for species and populations at risk of extinction and has been used successfully for generating mitochondrial DNA and microsatellite data. Despite its usefulness, DNA yield and quality obtained from buccal swab is usually lower than those obtained by more invasive collection methods, making many samples unusable. These lower yields can prove to be a limitation for several common methods requiring greater DNA yields. Numerous swab types are commercially available, with different shapes, sizes and costs. Swab characteristics can be important to take into account to ensure successful DNA recovery and the cost effectiveness of a research project. We obtained buccal swabs from common toads (*Bufo bufo*), using different swab types, and stored them in 100% ethanol or preservative buffer (composed of 10 mM Tris HCl, 5 mM EDTA and 0.5% SDS). In this presentation, we compare DNA yield (in ng.µL⁻¹) and quality (260/280 ratio) extracted from different types of commercially available swabs: (1) wooden swabs with large cotton tip, (2) Isohelix SK-3S (large flattened head), (3) Isohelix MS-02 (small flattened head), and (4) rayon-tipped MW100 fine-tip swabs. DNA was extracted using two methods, a QIAGEN DNeasy Blood & Tissue Kit, or McHale's salting out DNA extraction protocol. Highest mean DNA concentration was obtained from Isohelix MS-02 type swabs. Our results demonstrate that the type of swab used influences the yield and quality of DNA more than the extraction protocol, and we recommend taking this into account when doing research projects involving genetic sampling of amphibians.

Poster presentation

Prospects of ddRADseq-based hybrid zone approaches to decipher tropical anuran diversity: the case of Malagasy reed frogs (*Heterixalus*)

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DNA barcoding has revealed an astonishing number of deep mitochondrial lineages in tropical anurans, suggesting the possible presence of a huge amount of cryptic species diversity. However, population genomic studies have shown that mitochondrial data alone can be severely misleading, with phenomena of hybridization and mitochondrial introgression potentially confounding species-level lineages. A promising approach to assess whether genetically divergent and geographically adjacent populations constitute distinct species in the biological sense is the analysis of gene flow across hybrid zones, which serves as a proxy to reproductive isolation: in this approach, genetic admixture spanning over a wide geographic area would indicate conspecific populations. However, the required geographically dense sampling is rarely available for tropical amphibians. Here we take advantage of an exceptionally extensive sampling of Malagasy reed frogs, genus *Heterixalus*, a hyperoliid genus endemic to Madagascar, adapted to open areas outside forests. Gene flow across potential hybrid zones was assessed using genome-wide data generated by ddRAD sequencing in two species complexes: *H. betsileo* / *H. carbonei*, and *H. alboguttatus* / *H. boettgeri* / *H. madagascariensis*. Some of these species are very closely related, with genetic distances <1% in the mitochondrial 16S gene between *H. alboguttatus* and *H. boettgeri*. On the other hand, several species contain mitochondrial phylogroups that can differ up to 16S distances of >1%. Our genome-wide data did not reveal evidence for inter-species hybridization, which supports the present taxonomy. In addition, it identified distinct genetic clusters within all five species. Gradual admixture over relatively wide distances was particularly evident between genetic clusters of *H. alboguttatus*, *H. betsileo* and *H. madagascariensis*, suggesting that these clusters most likely represent intraspecific variation, and no further species-level splitting of these taxa is warranted.

Poster presentation

Spatial genomics of the *Vipera ammodytes* species-complex may help delineate Balkan subrefugia

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Despite often treated as a single refugium, it is now well-documented that multiple subrefugia integrate the Balkan region. Although their number and location is mostly unknown, phylogeographic analyses, complemented with past and present niche projections, are useful tools for their delineation. In this concept, we applied comparative genomic and niche analyses to study the spatial and temporal evolution within the iconic nose-horned viper species-complex (*Vipera ammodytes*) and explore the putative topographic and climatic agents that may have shaped its speciation.

We used ddRAD sequencing to build a time-calibrated species-tree and detect genetic clusters and evidence of past and current gene flow. We revealed a geographically structured genetic diversity with multiple hybrid zones throughout the Balkan Peninsula. Deep divergence corresponds well to old and imminent biogeographical barriers while two well-differentiated clades, currently lacking any evidence of gene-flow, are respectively distributed on the Northwestern and the Southeastern Balkans. In most other cases, speciation is incomplete with uninterrupted gene-flow detected in all possible contact zones. Despite the fact that most divergent subclades can be geographically referred to a putative glacial subrefugium disconnected from adjacent regions by extended glaciations, there is no evidence of ecological divergence. This suggests that speciation probably occurred in niche-pockets of analogous environments.

Comparing the genomic to the mitochondrial phylogeny and coupling genetic with niche analyses, we discuss (1) an evolutionary scenario that resolves all mitochondrial conflicts (2) the complex processes that have shaped the history of *V. ammodytes*, (3) the need for a taxonomic update that would better reflect the phylogenetic relationships within this complex and (4) the usefulness of combined genomic and climatic evidence as a tool to pinpoint the biodiversity hotspots for amphibians and reptiles in the Balkan region.

Conservation and population genetics

Poster presentation

The complete mitochondrial genome of the endemic *Pelophylax cerigensis* (Amphibia, Anura) and its phylogenetic position within genus *Pelophylax* (Amphibian, Anura)

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The Karpathos frog (*Pelophylax cerigensis*) is a Critically Endangered species according to the IUCN red list and is located on the island of Karpathos, South Aegean Sea. Utilizing a next generation sequencing approach, we obtained the whole mitochondrial genome of *P. cerigensis* in order to determine its phylogenetic position within the genus *Pelophylax* and to clarify the phylogenetic relationship between the populations of Karpathos Island and the populations from the island of Rhodes. High-throughput sequencing, using the Ion Torrent platform, generated 227,945±18,306 (mean±SD) reads averaged per sample. Reads of high quality were assembled resulting in the complete mitogenome of *P. cerigensis* of a total size of 17,922bp. Genome size and gene rearrangement are similar to other *Pelophylax* species, comprising 13 Protein Coding Genes (PCGs), 22 tRNAs, two rRNAs and one Control Region (CR). The overall mean genetic distance for the 13 PCGs ranged from 8.01% (COIII) to 11.6% (ATP8), while ratios of synonymous and non-synonymous substitution were <1 in all the 13 PCGs, indicating purifying selection. The phylogenetic relationships were investigated based on the concatenated sequences of 13 PCGs and 2 rRNAs (12S and 16S), using both Maximum Likelihood and Bayesian Inference analyses. Both analyses showed that individuals from Karpathos and the neighboring island of Rhodes define a well-supported monophyletic clade with very low genetic distance between them (p-distance=0.1%). Hence, individuals from Rhodes Island belong to *P. cerigensis* and not *P. bedriagae* as formerly thought, confirming the need of revision of its current conservation status and further examination of the *P. bedriagae* species group.

Session: Ecophysiology



Bombina variegata

Has fundamental niche shifted in the Aeolian *Podarcis*? An ecophysiological investigation

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While lizards are successful colonizers of Mediterranean islands, conditions of insular environments often differ dramatically from those prevailing in the continent in terms of food, microclimate, predation and density of conspecifics. Plastic/adaptive responses may result in shifts in fundamental niche. However, the magnitude of such shifts will depend not only on pressure intensity but also on the evolutionary timeframe. The Aeolian archipelago in NE Sicily is composed of volcanic islands erected during the mid-Pleistocene, but with a history of human colonization since the Neolithic. Of the two *Podarcis* species present, the endemic *P. raffonei* shares a Plio-Pleistocenic ancestor with the Sicilian *P. waglerianus*. The other, *P. siculus*, encompasses several Pleistocenic lineages in Italian Peninsula and Sicily, one of which colonized the islands after *P. raffonei* and possibly excluded it by competition from most of the Archipelago. We tested if these events affected the fundamental niche of Aeolian lizards by examining two ecophysiological parameters: preferred body temperature (Tp_{pref}) and evaporative water loss (EWL). We submitted adult males from four populations of *P. siculus* (Salina, Lipari and Vulcano in the Aeolians, Milazzo in Sicily) and one of *P. raffonei* (Capo Grosso in Vulcano) to two ecophysiological tests: 1) Tp_{pref} in a photothermal gradient; and 2) EWL in sealed chambers. *Podarcis raffonei* selected higher temperatures than *P. siculus*, while in the latter Tp_{pref} gradually increased from Milazzo to Salina. Remarkably, both species attained the most divergent Tp_{pref} in Vulcano. Neither interspecific nor intraspecific variation in EWL was recorded. Results indicate for the first time variation in thermal physiology between (sympatric) *Podarcis* and reveal conservativeness in hydric physiology. These outcomes are compatible with a biogeographic scenario of multiple colonizations of *P. siculus* in the archipelago. The subjacent mechanisms for thermal shift in insular lizards, including interactions with trophic ecology, should be investigated in deep.

Oral presentation

Limited body temperature variation in the *Vipera ammodytes* (Linnaeus,1758) during six years study

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Climate change would considerably influence survival of a species, especially of ectotherm ones such as snakes. We suppose that, if climate becomes warmer, both the costs and benefits of at least, thermoregulation would change for temperate-zone snakes. In modeling of the suitable habitats for *Vipera ammodytes* in central Serbia, we found that the most important factors associated with habitat suitability included habitat type and temperature seasonality. Therefore, we analyzed temperature data (cloacal temperature, substrate temperature, and air temperatures at both 5cm and 60cm above the ground) of 68 adult nose-horned vipers from two populations in central Serbia, measured during 2016-2021 in spring and autumn. There were neither interpopulation differences in cloacal temperatures, nor in substrate or air temperatures at site. For example, cloacal temperature varied from 20.6°C to 32.8°C with the mean of 27.23°C in the first, and from 22°C to 32.7°C with the mean of 27.42°C, in the second population. Sexual differences in cloacal temperature were not detected. Seasonal differences were detected in one population and only for air temperature at 5cm ($p < 0.05$). Although there were differences in measured environmental temperatures among some of the years of the study, cloacal temperatures differed significantly only in one population and only between the 2016. and 2017. Additionally, there was significant correlation between cloacal temperature and environmental temperatures (slopes were 0.50, 0.48 and 0.48 for correlation of cloacal temperature with substrate temperature, air temperature at 5cm and air temperature at 60cm, respectively with $p < 0.001$ for all three). These results suggest that *V. ammodytes* is a semi-thermoconformer, which is in concordance with previously reported diurnal and crepuscular activity.

Diverse like a Cocktail Bar – Snake Venom Proteomics of the Old World Vipers

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Known for their venoms, snakes have always fascinated and frightened humanity. Nowadays they constitute important resources for drug and antivenom development and are an intensively studied trait. The continuous analytical advances enable toxinologists to decipher venoms by modern omics technologies, so-called 'venomics'. In this contribution, members of the Viperinae subfamily are in focus for which an upsurge reporting on venom proteomes can be observed. These vipers are distributed in the Old World from Africa over Europe to Asia and face a variety of different preys and habitats. Therefore, the requirements for venoms and so their compositions are highly diverse. We generated the first comprehensive up-to-date database on Viperinae venom proteomes from the literature and differentiate between analytical methods and workflows. They were categorized into non-quantitative and quantitative approaches, with mass spectrometry as gold standard for the proteomic investigations. The overview and cross-study comparison for 89 Viperinae venom proteomes from the past two decades, revealed remarkable characteristics across the eleven genera at the intra- as well as interspecies level. But even of high medical relevant, most of the around 100 species are still unexplored at the venom level. In our ongoing studies, with a focus on Anatolian vipers, we utilize a combination of bottom-up and top-down strategies to get deeper insights into their venom complexity. Over the last year we extended our database with further snake proteomes of four genera, to inspect the previous observed taxonomic trends. For the future, particularly, the still large number of unknown venoms are a great opportunity for unexplored toxins and new insights into the evolutionary way of snake venoms.

Oral presentation

Thermobiological basis of cooperation in small heliothermic lizards of the genus *Darevskia*

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Reptiles are ectotherms and use external sources of energy to maintain body temperature. To do so, they exhibit special behavioral patterns, such as basking, behavioral cooling, using shelters and shuttling. These activities are time- and energy-consuming. However, thermoregulation should be effective and provide more energetic profit than cost.

Genus *Darevskia* is remarkable by comprising several parthenogenetic species. They are known to live in dense populations and aggregate during basking. We supposed that such cooperation enlarges total thermal inertia, improves effectiveness of thermoregulation and allows lizards to extend their period of diurnal activity. If so, cooperation must be most effective during evening basking, when insolation is too low to provide sufficient heat, but high body temperature is still needed for digestion after diurnal foraging.

To test this hypothesis, we performed a laboratory experiment. Animals were placed to glass terrariums 50 cm×50 cm×50 cm. A 50W incandescent lamp was used as a source of heat. We measured ambient body temperature with a UTi260B thermal imager, substrate temperature and air temperature with an AZ8803 thermocouple. Measurements were performed after the bulb was switched off until lizards' body temperature got equal to substrate temperature or all animals went to shelters.

We then performed GLMM with obtained data, treating specimen ID as a random effect. Both random intercept and random slopes were used and interactions between explanatory variables were considered.

There was a significant difference in expected body temperature ($p < 0.001$), but not in slopes of body temperature by environmental temperatures regressions (overlapping confidence intervals) between groups of two and three animals and single ones. Hence, we suggest that rock lizards do not obtain significant thermobiological profit from cooperation, at least during evening basking in groups of two or three specimens.

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Effects of artificial light at night on green toad development

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Amphibians, as the most threatened group of vertebrates, are susceptible to various anthropogenic influences. One of them is the presence of artificial light at night (ALAN) which is known to affect biodiversity and functioning of the ecosystems, including amphibians. In our research, we examined the effects of a two-week exposure to warm and cold LED light during the night period on the development of green toad, *Bufo viridis* embryos and tadpoles, and compared these data to the control group, kept under 12-12 hours of light/dark cycle. Each group was set in five replicates, with 20 eggs. No difference in the hatching time between the groups was observed. At the end of the experiment, tadpoles were measured (length and weight), and used for energy status data (total protein, lipid and carbohydrates content) and molecular biomarker analyses (acetylcholinesterase and glutathione S-transferase). Preliminary results suggest that ALAN affects tadpoles' size, and activities of biomarkers, while energy status was not significantly disturbed. These data provide important insight into physiological consequences that could arise from exposure to ALAN and more attention should be given to light pollution in urban areas where amphibians are present.

Oral presentation

Desiccation stress response of *Bombina variegata* tadpoles

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Many amphibian species inhabit temporary aquatic habitats for reproduction to maximize larval growth under favorable conditions but accelerate developmental rate to rapidly undergo metamorphosis under stress conditions when high desiccation risk exists. Corticosterone, one of the most important vertebrate stress hormone controls development, metabolism, and skeletal growth and together with thyroid hormone have a critical role in anuran metamorphosis under stress conditions. Here we compare the hormonal variation of corticosterone (CORT) in the yellow-bellied toad *Bombina variegata* tadpoles in response to different water levels (constant high-control, constant low, constant high+exogenous CORT) and the corticosterone inhibitor- metyrapone (MET) (treatment constant low water level+MET). Also, we investigated the effects of CORT and MET on life-history and morphological traits at metamorphosis. We found that *B. variegata* tadpoles cannot accelerate the developmental rate in response to pond drying. The constant low water level did not alter whole-body content of CORT and it did not significantly differ from high water level treatment with exogenous CORT. However, individuals from high water level with exogenous CORT have prolonged development, the highest mortality rate, the smallest body size and mass, and the widest but the shortest tails at metamorphosis. Constant low water level with MET decreased the whole-body content of CORT and it was significantly different between all groups, but it did not affect the developmental rate and tail shape although these tadpoles have intermediate size and mass between high water levels with exogenous CORT and control. We show that responsiveness to pond drying of *Bombina variegata* and its short larval period is probably associated with modified endocrine signaling pathway that control tadpole metamorphosis. Constitutively high whole-body content of CORT which was unaltered by pond drying leads us to conclusion that *Bombina variegata* development may be canalized but more research is needed.

Food for thought: Venom composition of *Vipera ammodytes* from the island and mainland population is likely related with diet

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Snake venom is a complex secretion of proteins, peptides and small compounds. Being closely related to feeding, this important ecological trait is highly variable. The resulting variations are notably characterised by the abundance of different toxins, both at inter- and intraspecific levels. Here we addressed variation in venom composition of two populations of *Vipera ammodytes* from North Macedonia. One population inhabits Golem Grad Island (Prespa Lake) where vipers feed mainly on ectotherms, and the other is from the nearby mainland (Konjsko village) where endotherms are included in the diet. Using the bottom-up protocol for venom proteome analysis, five pools (insular juveniles, subadults, adult males and adult females, and adults from the mainland) were analysed, from a total of 50 individuals (46 from the island and four from the mainland). The venom profiles of the insular and mainland population differed, likely reflecting prey divergence between the sites. At the population level, in Golem Grad, venom composition showed an ontogenetic shift in the relative occurrence of different toxin families. This change is probably due to the shift in the diet; juveniles feed mainly on centipedes and small lizards, while adults essentially consume lizards. On the contrary, there was negligible variation in venom profiles of island adult males and females, likely due to their similar diet. Our results reveal fine-tuned and rapid (ontogenetic) parallel shifts in diet and venom composition and highlight the need to consider species' ecology in any causal model of venom variability.

Oral presentation

Evidence that catecholaminergic systems are evolutionarily co-opted to mediate dynamic colour change during explosive breeding events in toads

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Communication in many species occurs through rapid colour change, where individuals dramatically alter their body colour within seconds or minutes. The physiological basis of these effects is poorly understood, although several studies point to the catecholaminergic systems as a potential mediator of rapid colour change in socio-sexual contexts. We study this possibility in explosive breeding toads (*Duttaphrynus melanostictus*), in which groups of males collectively change from brown to bright yellow during one- to two-day annual reproduction events. Thus, we test whether epinephrine (EP) and/or norepinephrine (NE) trigger colour change in sexually primed males. This seems the case, as both hormones mediate an increase in brightness and yellow colour saturation of the toads' skin. This effect supports the expression of a distinguishing sexual characteristic, which in turn underlies discrimination between males and females in large mating aggregations (>200 individuals). Accordingly, our results support the idea that selection might co-opt catecholaminergic hormone systems to help individuals quickly adopt a colour that optimizes behavioural interactions during intensive bouts of sexual competition. Endogenous stress apparatuses therefore appear to provide the mechanistic basis for an unusual and highly elaborate display to evolve.

Oral presentation

Can we use metabolic parameters to understand thermal preferences? A case study of five lacertid lizards

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We tested the Bogert's effect hypothesis which predicts that behavioural thermoregulation would uncouple physiology from thermal environment. We investigated three key physiological parameters related to metabolism in adult males of five lizard species with different temperature preferences (Tp) that were collected during fall 2020 in Portugal: haemoglobin concentration (HB), maximum rate of metabolic respiration (PMA), and antioxidant capacity (assessed by catalase activity – CAT and carbonyl concentration – CAR). Lizards were captured and acclimated in the laboratory under the same standard conditions for 1-1.5 months. PMA was measured at four temperature regimes - 28, 30, 32 and 34°C - that covered Tp range of all study species: *Podarcis virescens* (~29°C), *P. siculus* (~30°C), *P. carbonelli* (~32°C), *P. lusitanicus* (~33°C), and *I. monticola* (~33°C). Significant differences between species in HB, PMA, and CAR were observed, but not in CAT. One of the oxidative markers – CAR - correlated with HB and PMA. There was an expected positive effect of temperature on the rate of biochemical reactions in a range of 28-34°C (temperatures relevant to active lizards). The values of Q10 and Arrhenius activation energy (Ea), which illustrate the effect of temperature on the rate of biochemical reactions, showed that Ea was the highest in species that exhibit the highest and most precise thermoregulation. Therefore, our results deviate from the predictions of Bogert's effect and bring novel insights into the relationships between metabolic traits and thermal preferences in lizard species with different ecological requirements.

Poster presentation

Differences in the composition of *Vipera ammodytes* (Linnaeus, 1758) and *Vipera berus* (Linnaeus, 1758) skin secretions

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Snakes rely mainly on chemical cues for communication, and their pheromones are described as species-specific mixtures of mainly heavy, long-chained saturated or monounsaturated ketones, which might vary even between populations.

Here we investigated the compounds of skin secretions of *Vipera ammodytes* (Va, n=24) and *Vipera berus bosniensis* (Vb, n=10), with focus on ketone variety and frequency of occurrence. We separated the *V. ammodytes* samples into three groups considering three distinct populations in Bulgaria – Karlukovo village (eastern subclade of *V. ammodytes montandoni*, VamE, n=9), Kresna gorge (southern subclade of *V. ammodytes montandoni*, VamS, n=9), Balsha village (*V. ammodytes ammodytes*, Vaa, n=6), corresponding to three different clades, to test for intraspecific variations. The samples were analyzed and identified by coupled GC-MS. For each sample we recorded the presence or absence for each compound (n=52) that was found in at least one of the samples of the analyzed individuals with focus on the compounds which according to the literature most likely play a role in the intraspecific communication. We performed the Marascuilo procedure, to test whether a certain compound is more typical for any of the groups, and a Correspondence analysis to explore the relationships among the variables and observe the group differences.

Results revealed that squalene was not present in any of the *V. berus* samples while it was rather typical for all *V. ammodytes*. We also found statistically significant difference in the 2-pentacosanone frequencies between VamS and the Vaa individuals. The Correspondence analysis revealed that Vb semiochemicals composition is different than the typical for Va. It also shows that VamS and VamE are more similar than Vaa.

We hypothesize that the difference in ketones and squalene compositions in *V. berus* and *V. ammodytes* reflects an evolutionary adaptation for preventing hybridization. Our results also match the genus *Vipera* inter- and intraspecific phylogenetical relations.

Poster presentation

Differences in hypoxia/ischemia stress response in the Siberian wood frog *Rana amurensis* and the moor frog *R. arvalis*

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Most vertebrates cannot survive prolonged oxygen restriction. Among the exceptions are the Siberian wood frog *Rana amurensis* that can live for several months in anoxic water and the moor frog *R. arvalis* that withstands freezing up to -16 °C. Both conditions include the restriction of oxygen flow to organs. We compared 1H-NMR metabolomic data obtained for various organs of frozen *R. arvalis* and anoxic *R. amurensis*, as well as of control samples of these species. We found that both conditions involve accumulation of glycolysis end products and Krebs cycle arrest with the formation of succinate. Lactate and alanine were the dominant glycolysis products, with small amounts of ethanol for *R. arvalis*. In both species we detected the accumulation of 2,3-butanediol, which might also be an end product of glycolysis. Frozen *R. arvalis* accumulated large amounts of glycerol and glucose, the first case of both substances being used as low molecular weight cryoprotectants in the genus *Rana*. Unexpectedly, organs of hypoxic *R. amurensis* also contained glycerol, its role being elusive. Large amounts of nucleotide degradation products were found in frozen *R. arvalis* but not in hypoxic *R. amurensis*. We can conclude that the responses of *R. arvalis* to freezing and *R. amurensis* to hypoxia are similar in glycolysis upregulation and Krebs cycle suppression but differ in most other metabolomics aspects. This study was supported by the Russian Science Foundation (RSF) grant no. 21-74-20050.

Poster presentation

Hematocrit values and erythrocyte number in Mosor Rock Lizard (*Dinarolacerta mosorensis*)

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The Mosor Rock Lizard is an endemic species with a fragmented distribution in Croatia, Bosnia and Herzegovina, and Montenegro's Dinaric Mountains. We looked at the haematocrit and erythrocyte count in males and females from four Mosor Rock Lizard (*Dinarolacerta mosorensis*) populations in Croatia and Bosnia and Herzegovina. The haematocrit, or the percentage of blood made up of erythrocytes, reflects individual's physiological status. A specimen with a higher erythrocyte count absorbs oxygen more effectively, which can improve performance and likely raise an individual's chance of survival. Blood was drawn from the orbital sinus into heparinized capillary tubes 24 hours after the specimens were noosed and collected. After centrifuging the blood, the haematocrit and erythrocyte count were calculated. In July 2021, 97 blood samples were collected from four locations with elevations ranging from 600 to 1700 meters (Mt. Biokovo, 1300 – 1650 meters, Mt. Mosor, 880 – 980 meters, Mt. Visočica, 1680 – 1730 meters, and Suha Canyon 640 m – 820 meters). We were looking for statistically significant differences between Mosor Rock Lizard populations and sexes. Although erythrocyte counts vary from population to population, haematocrit values are constant. The highest values of erythrocyte counts are found on Biokovo Mountain, followed by Mosor Mountain, Visočica Mountain, and Suha Canyon. There is no significant difference between sexes for measured parameters when analysed collectively; however, when each location is tested separately, only population in Suha Canyon indicated a difference. Here, male haematocrit values were greater than female values in this study. This is the first data on the hemological values for the Mosor Rock Lizard and more research on this endemic lizard species will be necessary to comprehend the significance of the observed (dis-) similarities between populations.

Seasonal variation in ecophysiology of the lizard *Podarcis tauricus*

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During thermoregulation reptiles suffer evaporative water loss (EWL) and the intensity of water loss depends on the temperature and the humidity of the habitat. As evaporation increases with temperature, the EWL could constrain the activity of ectotherms when water is not available. The thermal preference of a species and its resistance to water loss can remain similar under different environmental conditions and over time, or they can change depending on a variety of factors. One of the most important factors regarding temperate climate is its seasonality, and ectotherms living in such climate may be adapted to the seasonal fluctuations in the environment and show different physiological responses depending on the season. During spring (May) and autumn (September) we observed two ecophysiological traits of the Balkan wall lizard (*Podarcis tauricus*): preferred body temperature (T_{pref}) with set-point range (T_{set}), and EWL rates (instantaneous evaporative water loss – $EWLi$ and accumulated evaporative water loss – $EWLa$). In these experiments we analyzed only adult males. Our findings showed that the thermal preference of *P. tauricus* was conserved between the seasons, as T_{pref} and T_{set} were quite similar in May and September (T_{pref} May: 21.5°C-36.1°C and T_{pref} September: 21.0°C-35.9°C; T_{set} May: 28.6°C-32.5°C and T_{set} September: 28.7°C-32.6°C). Overall hourly rates of evaporative water loss were lower in September and $EWLi$ was also with fewer oscillations in the autumn. Mann-Whitney U test showed a significant difference in the total $EWLa$ ($p < 0.05$). The analyzed lizards lost, on average, 2% and 1.2% of body weight in May and September, respectively. These results indicate that there might be a seasonal change in the sensitivity to water loss that enables the species to lose less water during the drier season.

Poster presentation

Voluntary thermal maxima of the *Vipera ursinii* complex determined by local adaptations instead of phylogenetic constraints

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Climate change is one of the biggest environmental issues. Its devastating large-scale impact on habitat degradation, subsequent loss of species and decrease of biological diversity has put climate warming to the fore front in conservation science. Hence, testing the temperature tolerance of ectotherms may never have been as relevant as now. The selected model system for our study, the *Vipera ursinii* complex, is composed of several species and subspecies of conservation concern, as the different taxa are threatened and listed as Vulnerable, Endangered or Critically Endangered in the IUCN Red List. Our study aims to answer the following general questions related to the *Vipera ursinii* complex: (i) Is the thermoregulatory strategy phylogenetically constrained? Or (ii) is there a thermal adaptation based on regional climates, leading to differences in tolerance of thermal extremes between taxa of these dwarf vipers? We have chosen this species complex as it includes taxa experiencing different climatic conditions and representing distinct phylogenetic lineages. We measured voluntary thermal maxima (VTM) of *Vipera graeca*, *V. renardi renardi*, *V. renardi eriwanensis*, *V. darewskii*, *V. dinniki*, *V. ursinii macrops*, *V. u. moldavica* and the Croatian lineage of *V. u. ursinii*. To detect phylogenetic signal in VTM we estimated κ and λ . To analyse individual variation of VTM we applied MCMCglmm using phylogenetic control. VTM of lowland taxa were significantly lower than of alpine-montane taxa. We did not detect a significant phylogenetic signal in VTM. The effect of annual mean temperature on VTM was significantly negative, whereas isothermality positively influenced it. The influence of snout-vent length on individual variation of VTM was significantly positive, while sex and the interaction of both variables had no effect. Based on these results, change in activity window could be predicted to assess the potential risk of climate change.

Poster presentation

Alpine salamanders from drier environments are more resistant to water loss compared to conspecifics from wetter habitats

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Desiccation risk may limit the activity window and distribution of terrestrial amphibians. Interspecific studies suggest that amphibians adapt to dry conditions through anatomical, physiological and behavioural changes, but whether water resistance correlates with desiccation risk at the population level is unclear. Here, we report on two experiments in which we determined water loss rates under different combinations of temperature and humidity in the Alpine salamander (*Salamandra atra*), a completely terrestrial amphibian with disjunct distribution. The study involved individuals originating from a warm, humid habitat (northern population) and individuals from a cold, dry habitat (southern population). In the first experiment, where animals could not control water loss behaviourally, salamanders from the northern population exhibited higher water loss than salamanders from the southern population, suggesting local adaptation or plasticity. In the second experiment, where salamanders were given the opportunity to behaviourally regulate water loss, individuals used this opportunity independent of their origin. Lab experiments revealed the relationship between water loss rate, temperature and humidity that were used to estimate water loss rates under realistic field conditions. The latter analysis indicates that desiccation risk may be a key factor determining the specie's activity window, and may have driven the increase in water loss resistance in the southern population.

Poster presentation

Invasive predator-induced chronic stress in common frog tadpoles

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Amphibian tadpoles show extensive phenotypic plasticity against predators. Among others, they reduce locomotor activity and develop smaller and earlier when exposed to predators. Measuring glucocorticoid hormones as the primary stress mediator associated with the stress response in vertebrates, provides a way to quantify physiological responses to stressors. When exposure to stressors is chronic, individuals experience the cost of reinitiating a glucocorticoids response and there is a change in the hormones of the hypothalamic–pituitary–adrenal axis such as glucocorticoids. In amphibians, this axis is the primary endocrine system controlling the physiological and behavioral response to external stressors via the regulation of corticosterone (CORT). The resulting physiological exhaustion occurs when CORT levels decrease because they can no longer be sustained. In our experiment, we wanted to test whether a new invasive type of predator of frog tadpoles, the slider (*Trachemys scripta*), induces a chronic hormonal response in common frog tadpoles (*Rana temporaria*). The whole body CORT-level of froglets of common frog was measured for individuals reared under permanent and temporal presence (i.e. tadpoles from Gosner stage 46) of caged slider. In both slider treatments, the tadpoles had a lower CORT-level than the tadpoles reared without it (12.6 ± 7.56 ng g⁻¹). Comparable CORT-level values in the permanent and short-term presence (6.8 ± 3.81 ng g⁻¹ versus 5.4 ± 2.52 ng g⁻¹) of the slider indicated that even short-term contact with the slider leads to a hormonal response at the level of chronic stress. Chronic stress is usually associated with persistently reduced CORT-levels, which can be detrimental and can adversely affect the growth and development of post-metamorphic individuals. The study was supported by a project of the GAJU 069/2022/Z.

Session: Introduced and invasive species



Pelophylax ridibundus

Introduced and invasive species

Oral presentation

Hybridization in crested newts (*Triturus*) at the outskirts of Vienna: limited introgression across a disrupted contact zone and sporadic introductions of non-native newts

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Crested newts (*Triturus*) are well-established model organisms in hybrid zone research. They are distributed across large areas of Europe and have come into secondary contact through postglacial expansion. Several molecular studies addressing these contact zones found different admixture proportions along transects ranging from bimodal to unimodal hybrid zones. We studied the contact between *T. carnifex* and *T. dobrogicus* in Vienna, Austria, which has been strongly affected by urban development, as well as a potentially allochthonous population nearby, combining molecular (3'UTR markers and ND4) and morphological approaches. We found limited exchange across the natural, but disturbed contact zone between *T. carnifex* and *T. dobrogicus*, and observed rampant hybridization between *T. carnifex* and an introduced species, *T. macedonicus*. Genetic markers suggest a Swiss (Ticino) or Italian origin of a few *T. carnifex* individuals. Morphological measures separate the two native species, but Viennese *T. dobrogicus* were more similar to *T. carnifex* than those from a reference population in Lower Austria.

Introduced and invasive species

Oral presentation

How to outcompete a native lizard? Violence is not always the answer

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At a time when we face a global biodiversity crisis, with a dramatic loss of species worldwide, it is intriguing how some organisms seem to be little affected by changes in their environment. They successfully adjust to different environments (such as invasive species) or to changes that take place in their native range (such as urban dwellers), and understanding how these particular species do this can be of major importance.

The Italian wall lizard, *Podarcis siculus*, has several invasive populations in different countries, and is also found living within cities across its native and invasive range. This lizard is thus a great model to investigate an array of questions regarding the success of species that can quickly adjust to changes in their environment. Moreover, it can also impact, displace and even eradicate native species. To test how *P. siculus* can outcompete a native lizard, we conducted a study with an introduced population in Portugal that lives in urban gardens, together with the native Green Iberian wall lizard, *Podarcis virescens*. Remarkably, instead of interference competition, we found exploitative competition occurring between the two species. Both species were tolerant of each other, but the invasive *P. siculus* was first to eat, consumed more food, and gained more weight than the native species over time. Our results indicate the behaviour of both the invasive and native species is likely to be promoting the invasive success of *P. siculus*, and further highlight the importance of investigating behaviour for understanding biological invasions.

Introduced and invasive species

Oral presentation

More time for aliens? Performance shifts lead to increased activity time budgets propelling invasion success

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In the Grinnellian niche concept, the realized niche and potential distribution is characterized as an interplay among the fundamental niche, biotic interactions and geographic accessibility. Climate is one of the main drivers for this concept and is essential to predict a taxon's distribution. Mechanistic approaches can be useful tools, which use fitness-related aspects like locomotor performance and critical thermal limits to predict the potential distribution of an organism. These mechanistic approaches allow the inclusion key ecological processes like local adaptation and can account for thermal performance traits of different life-history stages. The African Clawed Frog, *Xenopus laevis*, is a highly invasive species occurring on five continents. The French population is of special interest due to an ongoing expansion for 40 years and a broad base of knowledge. We hypothesize that the French population exhibit increased in activity time in the invasive European range that could be devoted to fitness-relevant activity and (2) tadpoles may have less activity time available than adult frogs from the same range. We investigate how thermal performance traits translate into activity time budgets and how local adaptation and differences in the thermal responses of life-history stages may boost the European *Xenopus* invasion. We use a mechanistic approach based on generalized additive mixed models (GAMMs), where thermal performance curves were used to predict the hours of activity and to compare the potential activity time budgets for two life-history stages of native and invasive populations. Our results show that adult French frogs have more activity time available in Europe than those in South African frogs, which might be an advantage in searching for prey or escaping from predators. However, French tadpoles do not have more activity time in Europe compared to the native South African populations indicating that tadpoles do not suffer the same strong selective pressure as adult frogs.

Introduced and invasive species

Oral presentation

Is there no place like home? Response of African Clawed frog tadpoles to novel environments

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The African clawed frog, *Xenopus laevis*, is an infamous global invader originating from southern Africa. In its native range, it can be found across two principal rainfall regimes and at different elevations. Geographic variation in adults has been observed for *X. laevis* throughout its native range. However, geographical patterns of tadpole phenotypes and the mechanisms that drive phenotypic variation at this life-history stage remain unexplored. We investigated the relative contribution of phenotypic plasticity and local adaptation to the tadpole phenotype of *X. laevis* originating from different climatic regimes and elevations. We present novel data on *X. laevis* tadpoles from two contrasting climatic regimes, showing that populations are both locally adapted and have an asymmetrical capacity to respond to novel environments. Additionally, we emphasise the invasion potential of *X. laevis* when sourced from these respective climatic regimes. Interestingly, all invasive populations present in Europe originate from winter rainfall climatic regime of South Africa with the exception of one population. The invasive population in western France originates from both the winter rainfall climatic regime and the summer rainfall climatic region. Thus, western France was invaded by *X. laevis* from two distinct climatic regimes. Here they rapidly expanded and admixed for ~40 years from a single known introduction point. Adults have been found to adapt to this novel environment, but here we also document how tadpoles in western France have adapted to their novel environment following admixture. Overall, we describe mechanisms whereby tadpoles can contribute to the invasion success of amphibians.

Oral presentation

Yellow-bellied toads in garden ponds: a successful habitat creation, and a support for long-term population persistence?

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The area of the intermittent Lake Cerknica, Slovenia, is home to 13 amphibian species/taxa, which take advantage of the diverse habitats, supported by the transient lake ecosystem. The lake itself holds only one population of the yellow-bellied toads (*Bombina variegata*), located in the outskirts, near the caves/sinkholes of Jamski zaliv. In the past, these habitats have been altered in order to facilitate water runoff or retention and toad's breeding sites have been partly destroyed. A surrogate habitat was therefore planned in a nearby location, elevated from the lake bottom and out of reach of floods and predatory fish. There, four prefabricated garden ponds were installed in 2018, filled naturally by rainwater and populated by yellow-bellied toads in the next spring. Monitoring of the toads using mark-recapture methodology started in spring 2019, and is still ongoing. We recognized individual animals by their ventral pattern and used programme MARK to estimate adult population size within seasons (closed models) and survival between seasons (open models). Besides adult yellow-bellied toads, we also detected egg masses, tadpoles and subadult individuals from the first season on. In 2019 and 2020 20 individual adult toads were captured, and 95% confidence interval (CI) of population size estimate was 20-24. In 2021 32 individuals were captured, and the estimate was 40 toads, with 95% CI 32-85. The cross-year open population analysis, showed a high and constant survival rate between years ($\phi = 0.981$) and temporally dependant parameter of entrance (pent1 = 0.0146; pent2 = 0.3349). In autumn 2021, a fifth pond was added, allowing to enhance the population. In May 2022 over 55 adult yellow-bellied toads were captured at every sampling occasion, but analyses are still to be performed. Nevertheless, it seems the habitat construction for yellow-bellied toads was very successful, but further monitoring will reveal the population persistence.

Introduced and invasive species

Oral presentation

Exotic supper: Exploring the diet composition of *Tarentola mauritanica* gecko in Madeira Island under a metabarcoding approach

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Trophic studies offer unique ecological insights, particularly important for insular systems, where predator-prey networks often include numerous endemic species. The Moorish gecko, *Tarentola mauritanica*, is a relatively recent introduction in Madeira Island, where it was first reported in 1993. In this study, we used a Next Generation Sequencing metabarcoding approach to uncover the invertebrate prey diversity present in the Moorish gecko's diet. Furthermore, we investigated how this introduced gecko might be affecting the endemic arthropods of Madeira and assessed if this introduced species might be consuming agricultural pests and arthropods that may act as vectors of human disease. The results revealed 250 different Operational Taxonomic Units in the diet of *T. mauritanica*, with 93 of them assigned to the specific level. 47% of the gecko's diet consists on invertebrates classified as introduced and 6% of them correspond to endemic species to Madeira. Moreover, among the introduced arthropods, 8 species are classified as agricultural pests. This study highlights the importance of trophic studies for monitoring introduced species in islands, considering their potential effects on the spread of agricultural pests and on the conservation of endemic species, emphasizing the potential of metabarcoding for assessing interspecific relationships in a non-invasive manner.

Introduced and invasive species

Oral presentation

Impact of invading species on biodiversity: diet study of the Green Whip Snake's (*Hierophis viridiflavus*, L. 1789) in Switzerland

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Next-generation sequencing is increasingly used in conservation biology to resolve complex interactions between species, either diet or gut parasites studies. We applied a recent long metabarcoding method to elucidate the Green Whip Snake's (*Hierophis viridiflavus*) prey consumption based on DNA extracted from stomach contents. Illegally introduced in Canton of Vaud (Switzerland), three populations of the Green Whip Snake have strongly developed in two regions, East (Chablais) and North. We suspect that this introduced species is threatening part of the local herpetofauna, especially the Asp viper and the Western Green lizard in this region. Consequently, an extermination program has been implemented from 2016 to mitigate *H. viridiflavus* expansion and its impact arising from its generalist diet. Stomach contents of 94 individuals removed from introduction sites were analysed. Our study revealed the consumption of 67 prey belonging to 9 species, primarily small mammals and reptiles. The recurrent presence of two parasitic nematodes was also discovered. Although cannibalistic behaviour could not be highlighted with this approach, a scavenging behaviour was suspected based on the presence of an insect used in forensic entomology (*Calliphora vicina*). These results confirm the opportunistic feeding behaviour of *Hierophis viridiflavus* and its ability to predate on threatened species. Although 86.6% of preys were not listed on the Swiss Red List, the impact on the Asp viper population can be important (up to 20% of consumed preys, which represent about 4 Asp vipers eaten per year and per Green Whip Snake) and could partially explain its strong decline.

Introduced and invasive species

Oral presentation

Where are you coming from? New data on introduced reptiles in Ukraine

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The increasing globalization allows organisms of the Earth unprecedented moving and areal changes. This is due to the food market, pet trade, or unintended transport. Ukraine is one of the least studied countries in Europe in terms of the distribution of reptiles, including alien or introduced species. In total, Ukraine has 21 native and at least five allochthonous species of reptiles. The most known introduced species is *Trachemys scripta*. Two species of lizards (*Darevskia armeniaca* and *D. dahli*) were deliberately introduced approximately half a century ago to the Zhytomyr Oblast. Recently, *Podarcis muralis* was found in the southern part of Ukraine, where was introduced probably from the Danube area. One species of gecko, *Tenuidactylus bogdanovi* found in Odesa city has been most likely introduced from Central Asia. In our study, we present data on three species of introduced reptiles for continental Ukraine with genetic affiliation to the population of the genus *Tenuidactylus* from Odesa. The first genetic data confirmed the affiliation of the Odesa population to *T. bogdanovi* which forms a separate genetic lineage different from the closely related species *T. fedtschenkoi*. In 2021 we found a specimen of *Pseudopus apodus* from Odesa in the museum collection. Although we have several hypotheses about the origin, one of them is this specimen was introduced most probably from the native range (Crimea or Caucasus). Completely unexpected are individuals of *Anolis* that were found in the Odesa seaport in 2021. We collected and genetically analyzed the DNA of two individuals and compared them with published data. We found that one individual is affiliated with *A. carolinensis*, particularly to the Gulf Coast/Inland clade and the haplogroup with samples from Wood County (Texas, US). The second individual was identified as *A. sagrei*, affiliated to the Southern Cuba clade that is also present in the introduced population from Florida where we suspect this introduced specimen came from.

Introduced and invasive species

Oral presentation

Predation patterns of invasive water frogs (*Pelophylax ridibundus*) in pond environments

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Invasive anurans are introduced worldwide, colonized large geographic areas and caused detrimental impacts to native ecosystems. The success of these alien predators as invaders is partly explained by their opportunistic generalist feeding behaviours that involve wide adaptative trophic niche and strong diet plasticity. From the point of view of native communities, this implies that a large diversity of native organisms may be impacted by predation in freshwater habitats. During the last decades, invasive water frogs (*Pelophylax ridibundus*) have been widely introduced in Western Europe but little is known about their potential impact on native communities through predation. In this context, we determined their diet in 21 ponds once a month over four months. Invasive water frogs exhibited generalist and opportunistic feeding strategies and fed on most of macro-invertebrate and amphibian pond communities. Most of the preyed organisms were terrestrial invertebrates but most of aquatic functional feeding groups were affected by predation, especially swimming predators. Despite not being the main prey of water frogs, some native amphibians suffered from high predation pressure during mating period by invasive water frogs given their high densities. Our results thus highlight one of the factors by which invasive water frogs may affect native pond communities and can pose a threat to native amphibian populations.

Introduced and invasive species

Oral presentation

Insight into the diversity of water frogs (genus *Pelophylax*) in Croatia and the cryptic invasion of Balkan water frog *Pelophylax kurtmuelleri*

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Species of European water frogs (genus *Pelophylax*) represent a complex group that is widespread in Eurasia, and numerous in the Mediterranean basin. They are characterized by a special mode of reproduction – hybridogenesis, which involves the production of fertile hybrids („kleptons“), such as *P. kl. esculentus*. European water frogs are known invaders examples of problematic amphibian translocations across Europe. Many taxa have already been introduced outside their natural ranges (such as *P. ridibundus*, *P. kurtmuelleri*, *P. bedrigae*, *P. bergeri*, *P. shqipericus*), mostly as a result of the food industry. The wide translocation of several species resulted in the co-occurrence of several alien species across many countries in Europe. As a consequence, intruders are competing with native species, progressively replacing their genomes. This kind of competition and elimination has been already known, probably the best example being *P. ridibundus*, *P. bergeri* and *P. kurtmuelleri*, eliminating *P. lessonae* in its habitat. Morphological identification of these species is difficult, especially when non-native taxa are present. In Croatia, *P. ridibundus*, *P. lessonae* and *P. kl. esculentus* are considered native. Additionally, two more species have been detected using molecular tools – *P. kurtmuelleri* and *P. shqipericus* as well as hybrids. Our preliminary molecular data show complex situation in Croatia, especially in its continental part. We detected widespread invasion of *P. kurtmuelleri* and limited introduction of *P. shqipericus*, with pure populations of native *P. lessonae* and *P. kl. esculentus* being scarce. This presents a serious ecological problem, especially for *P. lessonae* and *P. kl. esculentus* complex, which are readily replaced by the invasive *Pelophylax* species.

Introduced and invasive species

Poster presentation

Current distribution and introduction routes of *Podarcis* lizards into Poland

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Podarcis lizards are widely distributed European reptiles, which have often been introduced across the continent. In 2011-2014, three populations of *P. muralis* were discovered in the Strzelin Hills (SW Poland). Although a molecular analysis revealed that they belong to the Central European haplogroup I of the Central Balkan clade, the exact origin of the Polish populations could not be determined. The aim of this study was to assess the current distribution and introduction routes of *Podarcis* lizards into Poland.

In 2020-2021 *P. muralis* were searched for in 44 potentially suitable habitats (quarries, railways etc.) in the Strzelin Hills in the area surrounding the existing populations. No new sites of the wall lizards were confirmed.

Additionally, we conducted literature and online media review to assess the scale of accidental or intentional introductions of *Podarcis* lizards into Poland. Whenever possible, molecular analysis was performed to confirm species determination and its place of origin.

We found five events of accidental transport of lizards into Poland (*P. muralis* and *P. siculus*) or their escape from a terrarium (*P. muralis*). In two others, *P. muralis* and *P. siculus* individuals were intentionally released by the exotic animal breeder. At two introduction sites, the lizards formed populations existing for at least a few years. In the vicinity of Świdnica (SW Poland), *P. muralis* coming from Pula, Croatia were observed in 2017-2021. In Ustroń (S Poland), *P. muralis* coming from Stuttgart, Germany have been living on a private property for over a dozen years.

Our research showed that the occurrence of the common wall lizard in the Strzelin Hills is limited to three already known sites. From time to time, attempts to introduce *Podarcis* lizards into Poland are being documented. Although the newly formed populations do not show signs of invasiveness, further monitoring is necessary.

Introduced and invasive species

Poster presentation

Multi-criteria assessment of control methods for the invasive *Trachemys scripta* in Switzerland

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Invasive alien species (IAS) are a major cause of biodiversity loss, impacting endemic species via predation, competition for resources, habitat alteration and hybridization. They additionally can pose threats to human health and activities. There are numerous approaches to control IAS, but their success can vary widely depending on the target species and management context. A formal evaluation of different methods is therefore helpful, especially when dealing with limited resources and complex ethical implications of lethal and non-lethal control methods.

This study focuses on control methods for the pond slider *Trachemys scripta*, one of the 100 most invasive species according to the IUCN. They have been exported from the United States to Europe via the pet trade. The presence of *T. scripta* can have negative ecological impacts to native European pond habitats, particularly through competition with the European pond turtle (*Emys orbicularis*). In Switzerland the species already occurs and reproduces in several locations.

Our project assesses some common methods for *T. scripta* control in Switzerland, in terms not only of biological effectiveness, but also of costs and welfare implications. For effectiveness, we have built a population model to project *T. scripta* population dynamics under different control methods, using a mix of existing data from previous projects and novel data collected between the months of June and August. Based on the modelling results, we then estimate the total management costs for each method. Finally, for each control method we are collecting information about the physical conditions of turtle capture, transportation, and captivity; here we present preliminary results and the framework for this welfare assessment, using the well-established Five Domains of assessing animal welfare. We will then use this information to assess each trapping method by eliciting expert opinion during an organized workshop.

Through this comprehensive assessment, we aim to facilitate rational planning, balancing ethical and financial constraints against biological evidence, allowing optimal protection of native species.

Introduced and invasive species

Poster presentation

Invasion of the beauty rat snake, *Elaphe taeniura* Cope, 1861 in Belgium

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We report on the establishment of the beauty rat snake, *Elaphe taeniura* Cope, 1861, a large, oviparous colubrid native to Southeastern Asia, in Belgium. The snakes have invaded a railroad system next to the city of Hasselt in the northeast of the country (Limburg province), successfully reproducing and spreading. Our report is based on validated citizen science observations, supplemented with targeted surveys performed on site. The species has been recorded in the wild since 2006, most probably following an introduction linked to the pet trade. Genetic identification, based on the COI gene, confirms that the sampled individuals belong to *E. taeniura*. In addition, the snakes recorded in Belgium phenotypically match *E. t. taeniura*, a Chinese subspecies. So far, only the Taiwanese subspecies *E. t. friesi* was reported to be invasive and have an impact on endemic mammals and birds, in Japan. The exact date of introduction, spatial extent and population size are currently unknown, but the number of observations increased in recent years. Sightings exist from an area as large as 208 km², yet the core distribution is currently estimated to be no more than 2 km². Based on what is known on its ecology and distribution, we classified the species as a watchlist species with moderate environmental risk, currently occurring in isolated populations. However, the species' distribution and invasive potential in Belgium remain largely unknown, and a full risk assessment would require more data on its ecology. As the management of more widely established snake populations is notoriously difficult, we advocate rapid eradication as the most appropriate risk management strategy. As experience with the species is limited, this would require testing a combination of removal methods (hand capture, specific traps) in an adaptive management strategy. The conditions of an active railway, inaccessible to the public, pose a particular management challenge. Dedicated snake surveys to determine invasion extent are urgently needed to inform such response.

Session: Paleoherpetology



Zamenis longissimus

Poster presentation

Upper Pliocene to Middle Pleistocene snake fauna in Serbia in the light of climatic oscillations

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Climatic oscillations had a great impact on the distribution and evolution of various taxa, including snakes. Fossil snakes in Serbia have been identified from the localities of the Lower Miocene to Upper Pleistocene age. Here, we present fossil finds from Upper Pliocene to Middle Pleistocene localities and compare them with Upper Pleistocene finds and recent snake fauna. The locality Ridake is of Upper Pliocene age (MN16, approximately 3 Ma) and is located in western Serbia. The fossil fauna of Kamenjak quarry on the Venčac mountain (central Serbia) is situated at the Early-Middle Pleistocene transition (1-1.2 Ma). The data for Upper Pleistocene snake fauna came from six caves in eastern and southeastern Serbia. While the snake fauna of Upper Pleistocene cave deposits does not differ from recent fauna in Serbia, the localities Ridake and Kamenjak show significant differences in relation to both of them. At these two sites, taxa of snakes that today belong to the Mediterranean fauna (*Elaphe quatuorlineata*, *Malpolon* cf. *M. monspesulanus*, *Hierophis viridiflavus*, *Telescopus* cf. *T. fallax*) were identified. In addition, the extinct species *Zamenis paralongissimus* was identified in both localities. The warm climate of the Upper Pliocene explains the distribution of thermophilic snakes at the Ridake site, while the Kamenjak fossil association was formed in warm interglacial during the Early-Middle Pleistocene transition. In addition, fossil associations of both localities include taxa that were identified in Upper Pleistocene caves deposits and are also found in present-day fauna of Serbia, such as *Zamenis longissimus*, *Natrix natrix*, *Natrix* sp., *Coronella austriaca*, *Vipera ammodytes* and *Vipera* cf. *V. berus* complex. From Middle Pleistocene, Ice Age enters a phase of intensive increase in the volume of the ice sheet; the climate becomes colder, resulting in a shift of the Mediterranean taxa range. Tracking fossil remains allows for concluding about snakes' responses to past, as well as future, climate changes.

Session: Pathogens



Bufo bufo

Pathogens

Oral presentation

Optimization of heat-treatment against chytridiomycosis in *Bufo bufo* tadpoles

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Chytridiomycosis is an emerging infectious disease of amphibians, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). In the last fifty years it has led to drastic population declines and even extinctions globally. According to previous studies the critical thermal maximum of *Bd* is lower than that of most amphibian hosts, which provides the possibility to use heat-treatment against chytridiomycosis. Therefore, accurate information on the thermal ecology of the fungus could form the basis of an effective, chemical-free method of disinfection. The aim of our study was to determine the combinations of temperature and exposure duration necessary to clear *Bd* from tadpoles. We experimentally infected common toad (*Bufo bufo*) tadpoles with *Bd* and subsequently exposed individuals to one of four different temperatures (20, 26, 28 or 30 °C) for one of two exposure durations (4 or 6 days). We also tested whether treatments affected the length of larval development and the body mass of individuals. Initial prevalence was 100 % in the *Bd*-exposed group, but no animals died during the experiment. Treating tadpoles at 28 °C for 6 days, or 30 °C for at least 4 days resulted in decreased prevalence, while treating animals at 30 °C for any of the two durations also resulted in decreased infection intensity. Length of larval development was not affected by infection or by temperature treatments. Body mass was lower in non-infected than in infected individuals in all heated groups. Our results suggest that common toad tadpoles are not resistant, but tolerant to *Bd* infection, and that brief heat treatment for just a few days at moderate heat can effectively lower their *Bd* prevalence and infection intensity, but interspecific differences and potential malign side-effects also need to be considered.

Pathogens

Oral presentation

Tourist incursions predict chytrid load in amphibians from the pristine “Lost World”

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The remoteness and isolation of South American tabletop mountain (tepui) summits may protect against infections that underpin global amphibian declines. Increases in recreational pressure in such unspoiled destinations, and in isolated ecosystems globally, pose a poorly understood risk of wildlife disease introduction, especially in supposedly immunologically naïve communities. We here report the first observed chytrid infections in the Pantepui biogeographical region. Infections significantly correlate with proximity to the nearest basic tourist infrastructure in four endemic amphibians occurring on tepui summits and their slopes. Phylogenetic relationships and environmental context suggest a high risk of severe and irreversible population declines in unique, early branching amphibians. These findings advocate for an urgent control of recreational pressure in isolated, highly vulnerable ecosystems.

Pathogens

Oral presentation

Ectoparasitism in Polystomatidae (Neodermata, Monogenea): phylogenetic position and mitogenome of *Sphyrnura euryceae*, a parasite of the Oklahoma salamander

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Reptiles and amphibians are parasitised by several families of monogenean parasites including Polystomatidae, members of which principally infect chelonians and batrachians. Sphyrnuridae with its single genus *Sphyrnura* exhibits ectoparasitism on salamanders and was traditionally considered a sister-group to Polystomatidae based on haptor morphology. However, recent molecular work supported its inclusion within Polystomatidae, at an early diverging, yet unresolved, position in the clade of polystomatids that otherwise exhibit endoparasitism of batrachians. Resolving the phylogeny of Polystomatidae including the position of *Sphyrnura* is essential to understand factors driving the evolution of Polystomatidae including shifts between ecto- and endoparasitism. For instance, a sister group relationship between *Sphyrnura* and the other known polystomatid parasitising salamanders (*Pseudopolystoma dendriticum*), would indicate a tendency towards host-parasite coevolution. Records of *Sphyrnura* are scarce with phylogenetic studies only ever having included data from *S. oligorchis*. Detailed morphological examination and comparison with type material identified *Sphyrnura* worms infecting Oklahoma salamander (*Eurycea tynerensis*) as *S. euryceae*. Along with an amended diagnosis of *Sphyrnura* we provide the first molecular data for *S. euryceae* in the form of a mitochondrial genome and the nuclear (18S, 28S rRNA) and mitochondrial markers (cox1, 12S) with which we inferred the phylogeny of Polystomatidae. Our phylogeny identifies two clades within Polystomatidae, one infecting exclusively batrachians, the other mainly chelonians. Although not fully supported, *Sphyrnura* appears as early-branching within the former. As such, we consider Sphyrnuridae invalid. *Sphyrnura*'s apparent early-branching position indicates ectoparasitism as ancestral with endoparasitism having evolved later in the 'Polbatrach' clade. However, the reduced number of haptor suckers in representatives of *Sphyrnura* is a derived characteristic potentially resulting from paedomorphic evolution. There is a tendency towards phylogenetic congruence of polystomatids and batrachian hosts, albeit with some exceptions such as two independent colonisations of salamanders. Contrarily, polystomatid parasites of chelonians showed evidence of multiple host switches.

Pathogens

Oral presentation

Occurrence of chytrid fungus (*Batrachochytrium dendrobatidis*) and body condition in syntopic water frogs *Pelophylax shqipericus* and *P. ridibundus*

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We studied the presence of chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) infection in two syntopic water frogs *Pelophylax shqipericus* and *P. ridibundus* in a sample population from southern Montenegro. In total, 45 and 34 adult specimens of *P. shqipericus* and *P. ridibundus*, respectively, were tested for *Bd* infection. Here, the infection was confirmed for the first time in *P. shqipericus*, a vulnerable endemic species of the Balkans. There was a greater proportion of infected specimens in *P. shqipericus* than in *P. ridibundus* (15.6% and 2.7%, $p < 0.05$, respectively). Males and females did not significantly differ in *Bd* prevalence both within species or at genus level. Infection intensity was low in both species and body condition did not differ between infected and non-infected specimens. However, *P. shqipericus* had significantly lower body condition index than *P. ridibundus* in general, indicating elevated exposure to environmental stress in a comparison with congeneric species. The emergence of the pathogenic *Bd* fungus in the small-range threatened water frog species is worrying and indicates the need for close monitoring, urgent risk analysis, and population health assessment.

Pathogens

Poster presentation

***Pseudotaeniolina globosa* and *Quambalaria cyanescens*: Rare fungal species within the microbiome of green frogs' integument (*Pelophylax esculentus* complex) in Serbia**

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Fungi and bacteria are among the most frequently encountered microbiome constituents of the nutrient-rich amphibians' integument. Mycological analyses of the integument of the water green frogs from the *Pelophylax esculentus* complex were conducted in three localities in the region of the South Banat District where all three taxa (*P. ridibundus*, *P. lessonae*, and *P. esculentus*) coexist. Identification of fungal isolates was conducted by molecular methods, namely by amplification of the internal transcribed spacer region of ribosomal DNA (ITS) and β -tubulin (BenA) genes. After BLAST analyses, two rare fungal species: *Quambalaria cyanescens* (Microstromatales, Basidiomycota) and *Pseudotaeniolina globosa* (Capnodiales, Ascomycota), were documented. As a plant-colonizing fungus, *Q. cyanescens* is predominantly associated with *Eucalyptus* spp. in Australia. The presence of this fungus on *P. esculentus* in the locality Jaruga is the first record for amphibians, as well as the first record for Serbia. Although reported as a predominantly plant symbiont, *Q. cyanescens* is regarded as a potential opportunistic pathogen whose impact on amphibians has yet to be determined. On the other hand, *P. globosa* is a microcolonial, rock-inhabiting fungus seldom isolated worldwide, mostly on stone surfaces of historic monuments (eg., Djoser Pyramid in Saqqara, Egypt; the church of "Santa Maria di Mili" in Messina, Italy), and hence, has a limited dataset available in the GenBank database. The finding of *P. globosa* on *P. lessonae* from the canal DTD is also the first record of this fungus in Serbia as well as on amphibians. This finding is significant since *P. globosa* as a melanised fungus belongs to a large group of potential amphibian pathogens. In the case of *P. lessonae*, this is of particular importance since its global population trend is declining due to its constant and growing environmental threats (which include pathogenic fungi, e.g. *Batrachochytrium*) and consequently has a higher local extinction vulnerability than the other two taxa.

Pathogens

Poster presentation

Microhabitat specific performance of the chytrid fungus *Batrachochytrium salamandrivorans* and its impact on *Salamandra salamandra* (Linnaeus, 1758) in western Germany

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Nowadays, chytridiomycosis is one of the greatest threats to the diversity of amphibians worldwide. Caused by both, the well-known chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) and the chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*), it plays a decisive role in amphibian declines. The pathogen *Bsal*, which originally was introduced from Southeast Asia, was discovered in Europe for the first time in 2013. It is particularly harmful to caudate species, as it causes epidermal ulcerations, anorexia and ataxia, which ultimately lead to death in infected animals. As a result it has locally eradicated the formerly common fire salamander *Salamandra salamandra* in some regions of north-western Europe. In the Netherlands, for example, the fire salamander population has shrunk by 96%. Currently, Germany is the most affected area, with two hotspots: the Ruhr region and the southern Eifel. To simulate the performance of *Bsal* in its main invasive distribution area, we computed microclimatic surfaces, as temperature has shown to be the most important parameter affecting *Bsal*. These were simulated based on different habitat types, derived from the most recent Corine Land Cover 2018 maps. Although almost the entire study area would support the growth of *Bsal*, there are clear differences in the likely performance between different, small-scale environments. Based on these findings, possible *Bsal* hotspots are identified and recommendations for conservation activities are given.

Pathogens

Poster presentation

Major histocompatibility complex (MHC) variation and parasitism in lizard populations

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The adaptive evolutionary potential of a species or population to cope with environmental challenges is based on its genetic variation. Variability at immune genes, such as the major histocompatibility complex (MHC) genes, is a powerful and effective way to keep pace with diverse and rapidly evolving pathogens. To assess the relationship between the levels of variation at MHC genes, parasite burden, cell-mediated response and body condition, we characterized MHC class I exon 2 and exon 3 variation in different populations of the Balkan green lizard (*Lacerta trilineata*). We focused on island and mainland populations of *L. trilineata* that shows different levels of genetic diversity. Our findings showed that the levels of infestation and cell-mediated response are determined by the combined effects of habitat use and MHC diversity. Each factor had a distinct effect, with habitat use generally increasing infestation levels whereas too high MHC diversity imposing limitations on the efficiency of immune response and thereafter pathogen recognition and parasite burden. Confronted with the multitude of pathogens due to habitat deterioration, lizard populations with a large MHC allele reservoir are more likely to encompass individuals with resistance alleles to parasites. Yet, at the same time, at individual level, there is an urgent need to delete T-cells that react with self-peptide MHC combinations, providing a possible mechanism that constrains the expansion of MHC genes. Overall, our results deepen our understanding of the complex processes shaping host-parasite interactions.

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Pathogens

Poster presentation

Citizen Conservation - Upscaling ex situ conservation efforts

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The ongoing decline of biodiversity has been well documented over the last decades, but so far, efforts to stop it or even reverse it have been unsuccessful. Thus, despite numerous existing in situ and ex situ conservation efforts, additional endeavours are required. We believe that many ordinary persons already have the means and will to fight against biodiversity loss and can supplement current programmes, but these resources have to be activated. This might be especially true for ex situ conservation strategies where professional institutions and many private persons have already saved many species from extinction. Especially private persons can have the means to contribute to ex situ programmes for selected species but these efforts are rarely coordinated. With the contributions of private conservationists, we aim to increase ex situ capacities significantly. Citizen Conservation is an NGO under whose umbrella zoological institutions and private enthusiasts, in a joint effort, keep and breed endangered species of selected animal groups, currently amphibians and fish, following evidence-based management guidelines. We illustrate the framework of Citizen Conservation, the coordination guidelines of our programmes, that we have significantly increased ex situ capacities, and display our road for the future. In addition, we use the case study of the Fire salamander (*Salamandra salamandra*), which is under acute threat by the chytrid fungi *Bsal* (*Batrachochytrium salamandrivorans*), to show how such a programme could be implemented on a larger scale, for example in Germany, and how a concerted European effort could look like.

Session: Population, community and ecosystem ecology



Salamandra salamandra

Population, community and ecosystem ecology

Oral presentation

Atlas data reveal pattern and process of species replacement

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What good are distribution atlases? What is the point of an atlas for Europe, for a country, for a region, a department and a community? Due to uneven sampling and other biases most atlases are largely unsuitable for research into ecogeography, with as a notable exception the data for Portugal, thanks to the work of Rudolf Malkmus. Some analytical shortcomings can, however, be circumvented by taking a comparative approach. I here analyse published data for the newts *Triturus cristatus* and *T. marmoratus* at three nested spatial scales, namely the country of France, the region Pays de la Loire and the department Mayenne. Differential two-species distribution modelling highlights the parameters altitude and forestation as informative to the reciprocal *T. cristatus* – *T. marmoratus* distribution, along with temperature and precipitation. Soil parameters are not selected. Within the area of range overlap and hybridization the species distribution is a mosaic, with isolated occurrences beyond either species' contiguous ranges. This spatial configuration is suggestive of competition and species replacement, but who is replacing whom? How to get from pattern to process? I will present a variety of spatial and ecological arguments that suggest that *T. cristatus* has been expanding its range southwards across the west of France, at the expense of *T. marmoratus*, altogether suggesting that atlas data can be made to use after all.

Oral presentation

Brutal tortoise kismet: causes and consequences of extreme sex-ratio bias and insufferable sexual conflict

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Sexual coercion generally promotes mating success. Nevertheless, theoretically, under an adult sex ratio (ASR) heavily biased in favour of the coercive sex, sexual conflict can develop excessively and preclude reproduction, instigating a population collapse. This pattern has however, only been induced experimentally, but never observed in nature. We were fortunate enough to stumble upon a population of free-ranging animals, whose long lifespans allowed us to amass a capture-recapture dataset that slowly revealed they were indeed experiencing this peculiar demographic drift. Comparing two very dense populations (>100 ind/ha) of Hermann's tortoises with contrasting ASRs, promoting varying sexual conflict, we identified the cause of ASR-bias, assessed the costs to the abused sex, and estimated population fate. Namely, heavy ASR-bias (10.6), maintained by significant sex differences in annual survival between females (0.84) and males (0.97), induced pronounced sexual conflict, which consequently inflicted upon the coerced females: (i) low body-condition, (ii) intense cloacal injuries even during immaturity, (iii) small body size, (iv) proneness to risky behaviours (i.e., plunging from high cliffs), and (v) low fecundity. Variation in adult survival of such long-lived animals should be heavily canalized against environmental variation; nevertheless, sexual-coercion in this population generates frequent mortality events, which according to the high temporal-variance in female survival, cannot be buffered by behavioural or physiological adaptations. Overall, these female tortoises live a short and precarious life, and the number of recruits has likely declined over time and initiated an extinction vortex. This study not only fills gaps in ecological and evolutionary knowledge from a natural context, but for animals with environmental sex determination such as the Hermann's tortoise, such insight could be critical for their conservation in a globally changing climate.

Oral presentation

Assessing microhabitat preferences and population density using capture-mark-recapture method for *Telescopus fallax* (Fleishman, 1831) in central Dalmatia

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The Cat Snake (*Telescopus fallax* (Fleischman, 1831)) is the only species representing the genus *Telescopus* in Croatia. Characterized as a nocturnal species with the affinity for bare, rocky substrates its distribution is limited to warm, narrow coastland areas. The focus of this study is on its northernmost range of distribution, karstic river canyons, which are shown to be suitable habitats due to the uplift of a warm air. Over the course of 3 consecutive years 62 snakes were sampled. The species was never sampled in significant quantities thus no study describing ecology or habitat preferences was published beforehand. The study was focused on a capture-mark-recapture polygon established along a cliffside next to the city of Skradin and the west slope of Krka river canyon next to the Krka Monastery. Due to the illusive nature of this species this research was aimed at testing the functionality of the capture – mark – recapture method as a tool to assess the population density and consequently, attempting to describe preferred microhabitat conditions for this species. The research polygon size was 3,4 ha or 34.000 m². Out of the 55 snakes that were sampled, 18,75% were recaptures; 76% of sexed snakes were females and 24% males. Majority were adults (81%) with subadults and juveniles represented with 19%. The estimated population size, using the Cormac Jolly – Saber (CJS) model, is 5740±627 individuals for the polygon, or 1,7±0,2 individuals per 10 m². It was observed that they prefer rocky slopes with S to SW exposition where the stones are insolated during late afternoon hours. Further research is needed in order to fully understand the nuances of habitat preferences for *T. fallax* as well as a more in-depth approach to their population structure and distribution.

Oral presentation

Is trophic differentiation of green frogs in *Pelophylax ridibundus* - *P. esculentus* - *P. lessonae* population systems in South Banat, Serbia related to habitat suitability?

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Population systems comprising all three frog taxa from the complex are rare and in Serbia are recorded only along the Danube river. This study suggests green frogs' trophic differentiation from three localities adjacent to nature preserves with different levels of anthropogenic pressures in South Banat is related to their habitat suitability (HS). Diet and habitat suitability differences were recorded across all localities which have habitat characteristics conforming to typical green frogs' habitats. Samples from 221 adult frogs were obtained and a total of 1477 prey items were identified. Insects dominated the diet for all three taxa with 88% of relative abundance (Hymenoptera 28%; Coleoptera 18%; and Lepidoptera 17%), with 12% being other small invertebrates – spiders, crustaceans and molluscs. Rank abundance curves suggest that frogs acquired prey opportunistically, in line with previous studies and optimal foraging theory predictions. Low trophic differentiation was present with locality-specific prey dominating the ordination. Observed patterns were better explained by HS values than by taxon-specific characteristics. The locality Jaruga, with river-like features, surrounded by orchards and Nera river floodplain forests, had the highest HS value, with moth larvae being the dominant prey. A slightly lower HS was measured on the Stevanove ravnice locality, a mosaic landscape along the Danube river - open steppe-like pastures and hydrophytic vegetation under accelerated eutrophication where hymenopteran and dipteran prey dominated. The locality Danube-Tisa-Danube canal, a completely anthropogenically altered locality with a clover-planted causeway and severe tourist pressure, had the lowest HS values and dominance of beetles. Managers could use such results when devising new mechanisms of adaptive habitat management which optimize the presence and abundance of important species. Prey dependence on locality structure with different anthropogenic and zoogenic pressure heightens the importance of prey availability which frogs can acquire according to their habitat preferences and biotic constraints.

Oral presentation

Lacertid competition: Insights from mechanistic modelling

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Many abiotic and biotic aspects of the environment determine the survival of ectotherms such as lacertids. Specifically, many aspects of their lifestyle like, daily activity windows, time spent basking, searching for food and thermoregulation are heavily dependent on the thermal quality of the habitat and its temporal and spatial variation. Another important aspect that influences survival – through its influence in energetic consumption and allocations – are biotic interactions, the interspecific competition between members of the same ecological guild being amongst the most influential. *Iberolacerta horvathi* and *Podarcis muralis* constitute a pair of lizards belonging to the same guild often coming into interspecific interactions in syntopy. This species pair has previously been studied from different perspectives such as their ecophysiology, behavior, distribution and other elements of their life histories. The solid background of published data allowed us to adopt a mechanistic modeling approach to further understand the influence of the environment on their energetic allocation and activity patterns. We used a widely tested mechanistic model (NicheMapR) to integrate the predictions of biophysical models and metabolic theory (dynamic energy budget theory, DEB). We modelled 15 locations in southern Slovenia where these lizards naturally occur either in syntopy or allotopy and simulated the whole lifecycle of both species based on energetics taking into account environmental variability. That way, we estimated growth, development, reproductive output and energy allocation for both species under different conditions (in the absence or presence of competitor species). Our results are the first step towards building an integrative modelling framework using a mechanistic approach to study two species systems and their interactions and could in the future help scientists in modelling species distributions while incorporating species interactions. Results from such modelling may also be used further to understand the effects of temperature related climate change on interspecific interaction.

Oral presentation

Trophic niche of an epigeal population of *Speleomantes sarraabusensis*

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Speleomantes sarraabusensis is one of the eight European plethodontid species and is endemic to a small area in the south-east of Sardegna. The available information on its diet comes from a single population that can be observed in artificial springs, while no data exist for the poorly known epigeal populations. In this study we assessed for the first time the diet of an epigeal population of *S. sarraabusensis*, aiming to improve the knowledge on the trophic niche for this endangered species. We performed data collection on 25 March 2022 during night time. We actively searched for salamanders over an area of about 2,500 m². Captured individuals were photographed along with a reference card and attributed to different sex groups (juveniles, females and males). We used the program ImageJ to estimate individuals SVL from images. We collected the residuals from the last foraging activity of salamanders through the stomach flushing. The consumed prey were recognized at the order level and, if possible, considering specific families or ontogenetic stages. Using two generalized linear mixed models (GLMM), we assessed whether the number and the diversity (Shannon index) of consumed prey (the dependent variables in the respective GLMM) were affected by salamanders' size (the independent variable). Salamander sex was used as random factor. We collected dietary data from 33 individuals, recognizing 365 prey items belonging to 31 categories. The most consumed prey were Entomobryomorpha (25%), Diptera (9,2%) and Aranea (9,2%). No individual was found with an empty stomach. We found a significant effect of the SVL on both diversity and number of consumed prey; larger individuals consumed a larger number and more diverse prey. Our study provided the first data on the diet of epigeal population of *S. sarraabusensis*, contributing in expanding the knowledge on the trophic niche of this endangered species.

Oral presentation

Combining models and field observation to predict impacts of drought on temperate amphibians

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Climate change is already leading to an increase in extreme events, including drought. Drought is projected to increase in both frequency and severity even in temperate countries in Europe. One of the taxa most vulnerable to these changes is amphibians, as for most species water bodies are crucial for at least one life stage. Scotland provides an example of a temperate region that is projected to experience substantial increases in drought in the near future, with likely impacts on its amphibian populations. While some species such as *Epidalea calamita* are well adapted to summer drought and may benefit, the remainder of native species are likely to be negatively impacted. Using data on pond water levels and desiccation rate, we found evidence of drought during the breeding season (April-June) between 2014 and 2022. Our analyses combine drought models with field observation and suggest that drought is already a significant concern for amphibian conservation and resilience. Further, the changes are occurring sooner than expected and potentially turning otherwise healthy ponds, including those in nature reserves, into trap habitats for several species. We are now comparing these data to surveys from other regions in Germany, Spain, and the Netherlands, to find wider evidence of this issue across the continent. Conservation initiatives therefore need to take drought increases into account when designing interventions.

Oral presentation

Population size estimation of the Yellow-bellied Toad (*Bombina variegata*) at two locations along the Sutla River (Maceljska gora and Hum na Sutli), Croatia

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From March to September of 2019 and 2020, a survey of the Natura 2000 species *Bombina variegata* was conducted on Maceljska gora and Hum na Sutli. On Maceljska gora, we defined three microlocations. All three microlocation were situated mainly in the wheel-ruts, while the location on Hum na Sutli contains one small puddle and temporarily flooded parts of the forest along the stream. The individuals were captured manually or using an amphibian net, after which they were put in buckets. Then the sex and developmental stage of each individual was determined. After, we used the CMR method by comparing images of the captured toads' unique belly patterns using the I3S Pattern+ software. The population size was calculated using the MARK program. A total of 387 individuals were caught over the course of two years, with 147 of them being caught on Hum na Sutli and 240 on Maceljska gora. In the Hum na Sutli, the estimated *B. variegata* male population size was 38 individuals (95% confidence interval: 36-46 individuals) and female population size was 30 individuals (95% confidence interval: 28-38 individuals). The estimated male population size in the Maceljska gora, microlocation 1 was 34 individuals (95% confidence interval: 27-52 individuals), while for the female was 19 (95% confidence interval: 15-32 individuals). The estimated male population size in the Maceljska gora, microlocation 2 was 37 individuals (95% confidence interval: 30-54 individuals), while for the female was 32 (95% confidence interval: 26-52 individuals). The estimated male population size in the Maceljska gora, microlocation 3 was 34 individuals (95% confidence interval: 31-45 individuals), while for the female was 18 (95% confidence interval: 16-28 individuals). This is the first population size estimation of *B. variegata* species in Croatia and along the Sutla River region and we suggest that monitoring continues on these locations.

Oral presentation

Does climate crisis threaten Greek Amphibians? A species distribution modeling and time-series analysis approach

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Amphibians are the most threatened vertebrates in the world and the projected effects of climate crisis pose a new emerging threat for their populations. In the future, Mediterranean countries are expected to face unprecedented aridity, which may impact the overall biology of Amphibians. Such consequences may affect Amphibian range in a species-specific manner depending on the availability of suitable habitats. This study examines how changes in climatic conditions may shape the future distributions of twenty-one Amphibian species in Greece (4 endemic). We used species distribution models (SDM) to identify Amphibian species-richness hotspots in Greece, calculate the future range change and create habitat suitability maps for all species. We also used time-series analysis techniques, namely cumulative sums of anomalies, to detect possible impacts of temperature and precipitation on population sizes since 1979. Our results indicated an important reduction (95%) in the future distributions of the Amphibians that occur in Greece. Areas that are currently species-rich are predicted to be unsuitable to host Amphibians in the future, and these trends prevail throughout Greece. The largest average decrease in species richness seems to occur in the mountain ranges of Pindos, Rodhopi, and Corfu. We detected a significant change in temperature and precipitation conditions, with evident increasing trends in positive anomalies, occurring after 2006 until present. We also identified seasonal differences in climatic conditions among species of the Ranidae family. This is the first study assessing the effects of the undergoing climate crisis on Greek Amphibians. We anticipate that our results, combined with future research efforts, namely targeted collections at the suitable areas that were highlighted by our research, will assist in the conservation and better management of the Greek Amphibians populations, by taking into consideration any species-richness hotspots that are not included in the currently established Protected Areas Network in Greece.

Oral presentation

Factors influencing occupancy and reproduction probabilities in amphibians: an application of multistate occupancy models

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Correctly assessing the threats to metapopulation dynamics of amphibians is essential to effectively implement conservation measures. However, when dealing with amphibian metapopulations, the differences between actual populations, where reproduction occurs, and non-reproductive assemblages have been often overlooked. The occurrence of individuals and their reproductive success are two distinct phenomena that may be influenced by ecological factors in different ways, thus distinction between them species presence and actual reproduction is essential to correctly infer the threats for amphibian metapopulations. Multistate occupancy models allowed to classify the focal species' observed status during a survey into different presence states (for instance: absent, present with no reproduction, present with reproduction), and to correct the observations using the different detection probabilities of each presence state. We used static multistate multiseason models to assess the effects of local environmental conditions (area, permanence of the waterbody, sun exposure, aquatic vegetation, and forest cover) and of invasive predator species (alien fishes and *Procambarus clarkii*) on the occupancy and reproduction probabilities of 10 amphibian species, using data collected in 180 waterbodies in Northern Italy from between 2017 to and 2021. Our results showed that the considered parameters have different effects on occupancy and reproduction probabilities of multiple amphibian species. Sun exposure, for instance, had no effect on the occurrence of *Rana dalmatina* in our study area, but the reproduction probability of this species was higher in shaded environments. Moreover, we found divergent trends for occupancy and reproduction probabilities for the 2017-2021 period for three species. These results confirm the differences between occurrence and reproduction probability, that are differently influenced by the same ecological factors. Accounting for these differences is extremely relevant and should not be disregarded, since it may strongly affect our capacity to fully understand metapopulation dynamics of amphibian species and their declines.

Oral presentation

The diet of two sympatric salamanders, the Fire salamander and the Italian cave salamander

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The study of species diet is not only important for better understanding species requirements; it can also provide key information for designing appropriate conservation plans. The diet of species can be influenced by multiple factors, ranging from diversity and availability of prey (ecological opportunity), to the intra- or interspecific competition. In this study we present our preliminary results relating to the diet of two sympatric populations of the Italian cave salamander, *Speleomantes italicus*, and the Fire salamander, *Salamandra salamandra*. Studies focused on the diet of each single species already exist, but this is the first that considers sympatric populations of these two species. In April 2021 we made six surveys on rainy nights in a forest area of the northern Apennines, collecting the encountered individuals of both species. Captured individuals were photographed (for pattern recognition and to perform post-hoc measurements), weighed and subjected to stomach flushing, a harmless technique that allows residues from their most recent foraging activity to be inspected. We examined 314 individuals of *S. italicus* and 32 of *S. salamandra*, identifying a total of 3,088 prey belonging to 35 prey groups. The diet of the two species partially overlaps, but that of *S. italicus* shows a wider trophic spectrum including all 35 prey groups, compared to the 15 recorded that of *S. salamandra*. What has been observed could indicate that the trophic competition between these two species could be mitigated by both the size and the shape of the chosen prey. Indeed, *S. salamandra* seems to prefer larger wormlike prey compared to *S. italicus*, which mostly prey upon flying prey.

Oral presentation

Activity of the olm (*Proteus anguinus*) in surface habitats: ecological and evolutionary insights

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The olm is considered as a classic example of troglobiont organism. However, in the past different observations of individuals of the typical troglomorphic populations have been reported for springs of Venetia Giulia.

The aim of this work is to point out the non-random active use of surface habitats by the olm, providing a comparison with the occurrence observed in caves and performing an assessment of factors favouring ecotone habitats exploitation.

Since 2020 we started multiple day and night surveys of olms in both springs and caves. Each spring and cave habitat monitored has been characterised by respect to abiotic and biotic features, including planktonic and benthic prey availability.

We detected the olm at least once in 10 springs, with a maximum of 9 individuals occurring together. Detection probability in springs and caves was similar. Spring habitats provided higher density of potential prey available. Olms seems to prefer springs without predator fish and temporary hydroperiod. We recorded in one spring a larva of 3.5 cm which could be the smallest ever recorded in the field.

We suggest that epigean habitats and borders with surface may have an underestimated importance for animals adapted to subterranean environments, including the olm. Our results stimulate for testing if exploitation of ecotones between surface and groundwater can lead to differentiation in populations/subpopulations of stygobiont animals.

Oral presentation

A 12 years monitoring of *Trimeresurus stejnegeri* population during extreme climate events in northeastern Taiwan

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Extreme climate events such as excessive temperature, precipitation, prolonged dry and/or wet periods and tropical storms occurrence were recorded in Taiwan from 2010 to 2021. Particularly before and after the strongest El Niño event, the meteorological changes included the heat wave, drought, the occasional snow in the lowlands where it never occurred before, the change of frequency and scale of typhoons, the seasonal and sequential drizzle replaced by irregular short time heavy rain of annual precipitation patterns compared with historical records etc. This climate change may have influence on habitat quality deterioration and/or prey availability reduction of most vulnerable and declining snake species in Europe, Africa, and Australia, due to their small home ranges, sedentary habits and ambush foraging strategies. *Trimeresurus stejnegeri* is a medium size, sit and wait, semi-arboreal viper, with small home range, and is a common species in Taiwan. That makes it an ideal ecological model to determine how extreme climate events affect the viper population. Since June 2010, a long-term ecological monitoring of this species was conducted weekly in Yen-Wen experimental forest, National Ilan University, on a 635 m forest sampling trail, by visual encounter and mark-recapture methods. The study results indicated that the effects of prolonged drought and heat waves caused by the strongest El Niño within 2015-16 greatly reduced the relative abundance and survival rate of *T. stejnegeri*, up to 33.3% (RA) and 25.4% (SR) compared to the rest monitoring years in average. Besides, the space utilization of *T. stejnegeri* was shifted to the nearby riparian forest compared to the years without occurrence of extreme climate events. Higher temperature would enhance the activity of ectothermic predator (e.g., *Bungarus multicinctus*, *Lycodon rufozonatus*, *Protobothrops mucrosquamatus*) and increase the predation rate on *T. stejnegeri* in the monitoring area.

Oral presentation

Diverging niche dimensions in two syntopic lizard genus *Tropidurus* living in an area of the Caatinga Biome in Northeastern Brazil

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To investigate the divergence of ecological spatial niche dimensions we studied two syntopic lizard species living in an area of rock outcrops in the Chapada Diamantina of Igatu, municipality of Andaraí, state of Bahia, northeastern Brazil, an area of the Caatinga Biome, approximately 700 m.a.s.l. (12° 53'S, 41° 19'W). We evaluated if lizards *Tropidurus hispidus* and *T. semitaeniatus* used or selected structural (types of microhabitat and perch heights) and thermal resources (microhabitat temperatures and levels of sunlight) in ways that could ease living in syntopy. For each individual lizard we recorded 12 variables of the lizard and of their microhabitat. Data were analyzed in terms of divergence using Analyses of variance (ANOVA), one-way analyses of similarity (ANOSIM) and non-metric multidimensional scaling (NMDS). Most *T. hispidus* and *T. semitaeniatus* used rocks, resulting in a high overlap of structural niche dimensions. The former used such microhabitats according to the environmental availability and the latter selected rocks. *Tropidurus hispidus* selected shadier and colder microhabitats than *T. semitaeniatus* that selected warmer sites with full sunlight. *Tropidurus semitaeniatus* occupied regions further from vegetation shelters than from rock shelters. Larger *T. semitaeniatus* moved farther away from shelters than smaller individuals. *Tropidurus hispidus* perched higher than *T. semitaeniatus*. Distinctions regarding the use of spatial resources effectively reduced the overlaps of ecological niche dimensions, segregating the species and easing the life in syntopy. Congeneric syntopic species separate themselves by differing in components of spatial niche dimensions predominantly used, as *T. hispidus* and *T. semitaeniatus* living mainly on rocks, but diverging concerning levels of sunlight, microhabitat temperatures, and perch heights. The syntopy of the species was supported by their overlapping use of structural and thermal microhabitats. Nevertheless, each species has fine-scale divergences regarding components of their spatial niche dimensions.

Oral presentation

Spatiotemporal patterns of habitat use by the sand lizard (*Lacerta agilis* Linnaeus, 1758): Effects of climatic seasonality?

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The distribution and occurrence of a species in its habitat is inevitably linked with its ecology. To successfully monitor and protect species, it is important to investigate which species-specific factors influence its interactions with the environment. In this study, we focus on patterns in habitat use of the sand lizard (*Lacerta agilis*). Seasonal as well as sex and age dependent habitat use differences were reported from the species' range edges. To verify such trends in the core area of its distribution, we analyzed the habitat factors weather, microclimate, microhabitat structures and time dependence, which may have an impact on the behavior of the sand lizard. Using generalized linear models, hypervolumes, density estimations and Chi-squared tests, we found that the movement patterns of the individuals can neither be described by time differences, climatic conditions, or habitat composition, nor do they show habitat or weather-related differences of movement among sexes or age. Here we show that in the case of a population from the core distribution area at the Dellbrücker Heide (Germany), habitat use solely is influenced to a low degree by differences related to ontogeny of the sand lizards and does not depend on any of the other evaluated factors. These results from the core distribution area of the sand lizard show an enormous contrast to findings of populations from peripheral distribution areas, i.e. the United Kingdom, Latvia, Romania, Bulgaria, and the Pyrenees. This implies that seasonal habitats shifts are more extreme at the range edges of *L. agilis* to compensate deteriorating habitat conditions than in the periphery.

Oral presentation

Impact of climate change on the distribution of the island endemic *Natrix natrix cypriaca*

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The Cyprus grass snake (*Natrix natrix cypriaca*) is an endemic subspecies found in Cyprus. Despite being protected under national law and European legislation its distribution is still generally unknown. Recently new localities have been recorded, providing important insights regarding the ecology and distribution of the species. The present study uses the updated information to predict the species' current and future distribution at the Troodos Mountain range on Cyprus. At the same time it uses land cover to assess habitat connectivity and identify suitable environmental corridors.

Species Distribution Modelling (SDM) approaches are used, based on bioclimatic and biophysical environmental parameters, to predict the species' current and future distribution. Future distribution is being studied under an optimistic (SSP1-2.6) and a pessimistic (SSP5-8.5) climate change scenario. Moreover, Least Cost Path Analysis (LCPA) is employed to measure connectivity between the core areas of distribution for each climate scenario based on the identification of dispersal corridors during climate changes.

Our research showed a contraction of the species' distribution until year 2100, with a parallel shift towards higher altitudes. The knowledge on important ecological parameters related to the species niche has been enriched, while areas with limited connectivity have been detected. This research is the first attempt to predict the Cyprus grass snake's future distribution and evaluate the connectivity of its habitats on the Troodos Mountain. The outcome of this effort is an invaluable tool for the management of the species as it identifies areas of concern and is expected to assist with future conservation efforts.

Oral presentation

The effects of topography and climate fluctuations on the species and lineage richness of amphibians and reptiles living in the Balkan Peninsula

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The Balkan Peninsula is one of the most biodiversity rich areas in Europe, including many endemic species and evolutionary lineages of amphibians and reptiles. Due to the high topographic and climatic complexity, the Balkans served both as a speciation center and as well as an important refugium in the last millions of years. Consequently, the Balkans is an excellent area for the study of macro and microevolutionary processes. In this study, we compiled a dataset of occurrence records of every amphibian and reptile species in the area to calculate species richness in 50×50-km cells. To estimate richness of evolutionary lineages, we reconstructed species concatenated phylogenetic trees and using phylogenetic interpolations we split occurrence records into allopatric lineages which we then summed in the same defined area. We then compared species and lineage richness as a function of terrain ruggedness and two measures of past climate changes. These were defined as the absolute value of the difference between the current climate (annual mean temperature and precipitation) and the Last Glacial Maximum of the Pleistocene and the middle of the Pliocene, using linear regressions. We found that terrain ruggedness positively influenced species and lineage diversity in both studied groups. Climate difference from the Pliocene usually had a significantly negative effect on richness values, while the climate difference from the Pleistocene was not significant or also negative, indirectly supporting the importance of climate stability in richness. Our study suggests that a better understanding of phylogenetic processes can greatly help in interpreting currently observed patterns of biodiversity.

Poster presentation

The effect of burrow availability on the occupancy and abundance of the endangered Hungarian meadow viper (*Vipera ursinii rakosiensis*)

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The Hungarian meadow viper (*Vipera ursinii rakosiensis*) is a unique taxon living in the Pannonian Basin. Previous conservation interventions had no detectable effects on the abundance of the species, possibly due to the still-degraded suboptimal habitats and intensified predator pressure. The habitat selection of vipers is determined by several factors, such as vegetation structure, prey availability, or predator pressure. An overlooked but possibly important environmental source is burrow availability. These burrows are originally created by field crickets, ground squirrels, voles, and mice and later are utilised by snakes as shelter and hibernaculum. As burrow availability and diversity can be limiting factors in population growth, we examined the effects of these on the occupancy and abundance of the Hungarian meadow viper in the Kiskuns  g region (Hungary) using temporally and spatially replicated counts of vipers and burrow densities sampled across four sampling seasons in 2020-2021 (spring and autumn). We used multi-seasonal occupancy and n-mixture models in a Bayesian framework, where the environmental (operative) temperature was the explanatory variable for detection, while burrow densities (within three types) and diversity index of burrows were included as explanatory variables for the state (latent occupancy/abundance). The results of both models showed a positive effect of burrow diversity on viper occupancy and abundance, while burrow densities had no effect. The results suggest that there is no limitation in suitable burrows at the study sites, however, further studies should assess the influence of individual burrow occupancy by their original inhabitants, while future conservation research and efforts should focus on other parameters and potential threatening factors which could affect viper populations.

Poster presentation

DNA metabarcoding for the assessment of geographical differences of consumed algae in the Galápagos marine iguanas (*Amblyrhynchus cristatus*)

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Marine iguanas are endemic species of the Galápagos Archipelago where they inhabit all major islands as well as smaller islets. For most islands each marine iguanas population is characterized by one genetic cluster, except for San Cristóbal Island, where two subspecies (*Amblyrhynchus cristatus mertensi* and *A. c. godzilla*) are present.

The marine iguanas are primarily associated with the marine environment. They are the only lizards worldwide that forage on marine macroalgae, showing special nutritional adaptations. The consumed algae species have been identified previously only by direct observations during feeding activities and microscopic identification in faeces samples.

In our study we use a newly established and non-invasive DNA metabarcoding approach to identify consumed algal species from the faeces of marine iguanas. We developed primers for the ribulose-bisphosphate carboxylase (rbcL) gene and for the nuclear ribosomal 18S gene and applied a metabarcoding approach to individual faeces samples collected in representative sites from 11 islands of the archipelago.

The first results of our study suggested that the trophic niches between the two subspecies found in the San Cristóbal Island differ with respect to consumed macroalgal taxa and also indicates a clear preference towards red algae as food item. Despite the number of consumed algal species did not differ between the two subspecies (OTU richness; $P = 0.383$), diet overlap level between *A. c. mertensi* and *A. c. godzilla* was low (Schoener index = 0.345), suggesting that both subspecies consume different algal species in their natural environment.

Further analyses of our collected data will reveal if the subspecies of other islands also differ in their foraging niche and whether such difference of consumed algae reflects disparities in the abundance of algal species between habitats, or whether iguanas of genetically differentiated subspecies prefer distinct algal species.

Poster presentation

More than a decade of monitoring population abundance in three anuran species from Central Serbia

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Amphibians are the most endangered of all vertebrates; therefore, maintaining a long-term monitoring of local populations is important even for the widespread species. Neglecting the research on common species might result in many silent local extinctions. The negative effects of environmental changes can be reflected in a reduction of a population's size, with a strongest impact on species that reproduce during early spring (like *Bufo bufo* – common toad and *Rana dalmatina* – agile frog), although other species (*Pelophylax esculentus* complex – green frogs) could also be affected. Decrease in size of common toad populations was recorded in parts of Great Britain, the Czech Republic, Italy and Switzerland, while agile frog populations have usually been reported as stable. Declines in population size of some green frog populations were reported in parts of Poland and Turkey.

These three taxa were monitored from 2011 to 2021 in a permanent pond near village Zuce, at the southern outskirts of Belgrade (Serbia). Population size of the common toad seems to be declining over the years (Kendall's Tau = -0.60; Z = -2.57; p = 0.01). Number of females was constantly low, which raises concern. Fluctuation in population size was registered for both agile frogs and green frogs, with 2021 being the best year as most egg clutches and individuals were spotted. Density dependence effect on population size was confirmed for the agile frog (Kendall's Tau = -0.56; Z = -2.09; p = 0.04). Within a year, the activity of green frogs was increasing over the months until May/June and then it was decreasing until December. The largest number of individuals was spotted in May. There was a slight inversion in that decline in September, when maximum and minimum numbers of individuals were higher than in August. There were no correlations among the species regarding population size fluctuations.

Poster presentation

Initial estimation of lizard population densities on the selected islands of the Lastovo Archipelago, Croatia

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On several islands in the Croatian archipelago of Lastovo, it has been observed that introduced mammal species might have an impact on native species populations. One of the invasive species found on the islands is the Black rat. Rats are predators, and evidence from other parts of the world are conflicting, some research indicates that rat predation may be the cause of the population decline of lizards and others that rats do not have influence on lizards. Rat eradication is planned on some islands of Lastovo Archipelago as part of LIFE Artina - "Seabird Conservation Network in the Adriatic" project (LIFE17 NAT/HR/000594) and with this study we wanted to make an initial assessment of the lizard population densities on them before eradication. Two field trips to the Lastovo Archipelagos were made in May and June 2019 on selected 14 islands. Islands range in size from 0,35 to 47,42 hectares. During three field visits to each island, a linear transect with a length of 500–1000 m was established, where lizards were observed twice in a single day. Distance sampling was used to estimate the density, and each specimen's perpendicular distance was measured. Each island's data from the transects was analyzed separately with the program Distance 7.3. A total of 4164 adult individuals, including 2287 Dalmatian Wall Lizard, (*Podarcis melisellensis*) individuals and 1877 Sharp-snouted Rock Lizard (*Dalmatolacerta oxycephala*) individuals, were observed. Both species were recorded on all the islands visited. The density of the *D. oxycephala*, is 22 - 282 individuals per hectare, while the *P. melisellensis* is 28 - 251 individuals per hectare. These numbers represent initial values, and this assessment must be repeated following eradication to compare the situation before and after so it can be determined what kind of influence will rat eradication have on lizard populations on islands of Lastovo Archipelago.

Poster presentation

The lateral line – a reliable way for software assisted individual identification for *Hyla arborea*

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Traditional mark-recapture methods for amphibians and reptiles involve the application of invasive physical markers – from paint marking to toe clipping. During the past decade, there are a growing number of studies investigating the potential of using natural colouration in photographic identification methods, which allow for inexpensive, reliable and non-invasive identification. The relatively small species of the Hylidae family are susceptible to toe clipping, as it interferes with their climbing ability – so dorsal, ventral or leg patterns have been used in studies on different hylid species.

We tested the usefulness of the lateral line in the European treefrog *Hyla arborea* in software-assisted image recognition. A total of 143 adult treefrogs from a pond near the village of Oshtava, SW Bulgaria, were captured by hand in six sessions throughout April 2022. The right side of each frog was photographed and all animals were released at the site of capture after each session. Images were loaded into Hotspotter – free software for image recognition of animals. The region of interest used for comparison between the images was set as close around the body as possible to minimize external factors, but at the same time to allow all elements of the lateral line to be clearly visible. Results revealed a total of 27 recaptures, including five cases of multiple recaptures (three recaptured twice and two recaptured three times). All recaptures were suggested as the first potential match, and all but one of the multiple recaptures were ordered consecutively. Careful manual verification confirmed the program results, and there were no false positives (match scores for confirmed recaptures were >3 times higher than the respective scores for non-recaptures). Our results demonstrate that the lateral line would allow for a reliable and time-efficient individual identification of European treefrogs.

Poster presentation

Software-assisted individual image recognition of *Vipera ammodytes* (Linnaeus, 1758) – advantages and limitations

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In recent years, computer-assisted individual recognition of animals based on their morphological characteristics has become a valuable tool for ecological research. Still, reptiles are understudied in this regards. In particular, traditional marking methods are ineffective with snakes, as they lack limbs for toe-clipping and shed their skins (paint markings only last for a limited time). While they do have unique scale and colour patterns, manually comparing photographs is often very laborious, especially in large samples.

We tested whether Hotspotter – a specialized image recognition software that has been very successful with amphibians – would be able to recognise individuals of *Vipera ammodytes*. A total of 48 images of 13 individuals from three distinct populations were tested in two separate series, based on the “region of interest” used in Hotspotter – 1) colour pattern of the head and neck (“back”) and 2) frontal shape and scale position of the horn (“horn”). For both series, the program suggested six possible matches for each image, ordered by similarity score. All recaptures were manually verified a priori by A.D. and subsequently randomized, so during the testing S.L. did not have knowledge of the snakes’ origin and only relied on the images. Results for the “back” series proved to be inconclusive – while there were some very high-score matches (i.e., the first suggested matches were indeed recaptures), most images were ordered randomly. However, results for the “horn” series were very clear, with the software successfully identifying all seven recaptured individuals (incl. two cases of multiple recaptures with injury-induced changes in the shape of the horn). The main advantage of this method is that it drastically reduces time spent on identification. Still, it is advisable to have images of both the back and the horn, which can be compared in case of injuries.

Poster presentation

Results of a standardized snapshot of the *Trimerodytes percarinatus* population monitoring in a montane lake in northeastern Taiwan

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Water snake is a restricted and dominant predator and with obvious correlations to its hosted wetland trophic level and hydrological fluctuation. To monitor a remote montane wetland ecosystem dynamics via *Trimerodytes percarinatus* population, a standardized snapshot of ecological monitoring was developed and utilized since July 2019 in Song-Lo Lake, northeastern Taiwan. Song-Lo Lake is a high water-level fluctuation montane wetland, primarily driving by typhoon and heavy precipitation during summer and monsoon in winter and in spring. That exhibited annual characteristics on the physical condition and reproduction of *T. percarinatus* herein. Quadrotron UAV (unmanned aerial vehicle) was utilized for the real-time shoreline record and the waterbody area estimation. Water-level data loggers were installed and provided reference depth of the lake. Single mark and recapture procedure was used for the snake population study, respectively the first capture and mark in mid-July and then recapture in mid-August during the study years. Snakes sampling by the visual encounter method along the shoreline and shallow water areas were located by GPS. Collected snakes were measured SVL, TL (both in cm) and BM (in g), and also were examined if the scars on the body and the tail-stub were present or not. We used gentle palpation to determine the stomach contents condition and counted the clutch size for gravid females. Finally, we checked the presence of the PIT tags, and released it to the original collection site. The detailed results will be presented in the poster.

Poster presentation

Population characteristics of European pond turtle on Ludaš lake in Serbia

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Ludaš lake is one of the few natural lakes in the Vojvodina province, with a water surface of 328 ha. It hosts a population of the European pond turtle (*Emys orbicularis*), the only autochthonous species of freshwater turtle that is inhabiting Serbia. This is an open population, connected by the canals to other adjacent (sub)populations. Such a well-established network of artificial and natural water bodies in theory might interconnect many populations of the European pond turtle in the Pannonian plain, most probably forming a metapopulation system. For the purpose of the 10 years long and still ongoing population study (Capture-Mark-Recapture), only the north portion of the lake was investigated, with an approximate surface area of 9 ha. During this study, 820 capture events occurred, amassing 397 marked individuals in the study plot. The Schnabel index for estimating population size shows that the number of individuals in the study area is 596.7. A rough estimate of population density is 66.33 individuals per hectare. The adult sex ratio is close to 1:1 (201 females vs. 188 males), which is considered very balanced. A portion of caught juveniles is only 2%, and this is most probably due to the capturing method used (baited funnel traps, which are not suitable for capturing juvenile turtles). Continuous monitoring of population parameters such as population size and sex ratio can provide useful insights into the current state of the population and can be the firm basis for future conservation actions in the areas where and when some of these parameters come out of balance.

Poster presentation

**Spatial patterns and the climatic determinants of the temporal neck spots,
dorsal pattern polymorphism in European grass snakes**

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European grass snakes (*Natrix natrix*, *Natrix helvetica*, and *Natrix astreptophora*) are highly polymorphic natricid snake species widely distributed in the Western Palearctic with significant variations in the colors of their dorsal patterns as well as the shapes present on their backs. Most specimens of European grass snakes have a characteristically yellow, orange, white, red, or absent temporal spot behind their head that, in laboratory studies, was linked to the temperature regime experienced during the egg's incubation. Phenotype data derived from citizen scientists' data-sharing platforms such as iNaturalist are a great resource to test the link between the broad-scale phenotype distribution patterns derived from such platforms and the environment. Here, we scored 1585 specimens of European grass snakes (33 of *N. astreptophora*, 417 *N. helvetica*, 1135 *N. natrix*) shared on citizen scientists' data-sharing platforms over their entire range of the studied species to investigate the relationships between the temporal neck spots and their dorsal color and shape patterns and the broad temperature and precipitation trends. We extracted temperature-based bioclimate variables associated with each observation to create temperature profiles for each phenotype corresponding to the color of the snake's temporal neck spots and their dorsal color and shape patterns. We found a robust geographic clustering of the phenotypes following a similar pattern to the one described in the laboratory studies, where individuals from warmer climates have white or absent temporal spots behind their heads and individuals with red or orange temporal spots in colder climates. We also found that snakes with darker colors on their backs are more likely to be found in colder areas, while lightly colored snakes are found in warmer areas. We also emphasize the importance of combining data obtained from the citizen scientists' data-sharing platforms with laboratory or experiments in studying large-scale hypotheses.

Poster presentation

Trends in the abundance of predators of Hungarian meadow viper (*Vipera ursinii rakosiensis*) and the effect of grassland utilization on predation pressure

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The Hungarian meadow viper (*Vipera ursinii rakosiensis*) is a globally endangered reptile with a few sub-populations remained after habitat alteration and fragmentation. In Hungary, all known habitats are located inside nature conservation areas and significant practical conservation efforts were implemented via habitat reconstruction and development, ex situ breeding and reintroductions. Nevertheless, the estimated overall population size of Hungarian meadow viper is still very low, and the impact of conservation interventions is virtually immeasurable according to low densities. It is hypothesized that predation is the main factor influencing viper abundance. In this presentation, we aimed (i) to compile a list of all known predators of this particular viper subspecies and (ii) examine populations trends of these predator species around the viper habitats. We aimed (iii) to monitor the early effects of game species management implemented by Kiskunsagi National Park Directorate within the frames of by LIFE18 NAT/HU/000799 project and (iv) to study the effect of grassland utilization type on predation pressure. We observed 14 bird and mammal species preying on Hungarian meadow viper. All these predator species showed significant increase in their abundance partly due to game management and conservation success. Since in the new LIFE project we started intensive trapping numbers of hunted predators (Fox, Badger, Hooded Crow) reduced by 48.6% and the grassland uprooting by wild boar were significantly reduced by four-line electric fence. Using plasticine snake models at hayfields in a before-after mowing sampling design with extensively grazed control sites and we found that at the grazed grasslands (controls) the predation pressure was significantly higher than in the hayfields. The predation pressure showed a limited increase after mowing. To enhance the growth of the Hungarian meadow viper population effective predation control is most likely an absolute necessity.

Poster presentation

The feeding habits of the Spadefoot toads (*Pelobates* spp.)

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We performed a comprehensive analysis of the diet of two species of spadefoot toads (*Pelobates fuscus*, n=127, and *Pelobates syriacus*, n=304). Juvenile and adult specimens were collected from localities in Serbia, Macedonia and Bulgaria between 1975 and 2000. Prey analysis demonstrated qualitative and quantitative dominance of invertebrates in the toad diet. The diet consisted of easily accessible and abundant prey, placing both species into the group of non-selective predators. A single vertebrate specimen (Reptilia, Lacertidae) was recorded in the diet of *P. fuscus*. Although the presence of vertebrates in the diet of *P. fuscus* has not been reported previously, our finding confirms that vertebrates are a very rare prey for this species. Indices based on importance of certain food component were calculated to estimate the niche breadth and overlap for the two species, and to perform the comparative analyses of the diet through the seasons. *Pelobates fuscus* had the least varying diet during spring indicating a narrower niche and maximal specialization, and most varied diet in autumn, ahead of winter dormancy. *Pelobates syriacus* diet was most varied in summer, correlating with prey abundance, and its niche breadth varied less over the three seasons compared to *P. fuscus*. The greatest overlap of trophic niches for the two species occurred in the spring, and the smallest overlap in the summer, but the average overlap across all seasons was large, almost 94%. Our data do not support a significant role of syntopy and allotopy in feeding habits of *Pelobates* spp.

Poster presentation

Toad-headed agama (*Phrynocephalus mystaceus mystaceus*, Pallas, 1776) on the sandy massif of Sarykum: directions of population research

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Toad-headed agama (*Phrynocephalus mystaceus mystaceus*, Pallas, 1776) is a widespread species of sandy deserts and semi-deserts of Russia, Kazakhstan, Central Asia, Northeast and East Iran and Northern Afghanistan. On the territory of Russia it is included in the Red Books of the Chechen Republic and the Republic of Kalmykia with status 1 as a species that is endangered; in the Red Books of the Astrakhan region and the Stavropol Territory with status 3 as a rare species and in the Red Book of the Republic of Dagestan with the status 2 as a species that is declining in numbers. The population of this species inhabiting the sandy massif of Sarykum is of particular interest. These sands are a typical example of ecological isolation or in the broad sense of the "island habitat". The study of such populations at the moment is of particular importance, since for many species the "island habitat" is becoming an increasingly characteristic distribution variant. There is another side that requires attention to this species and other psammophiles of the territory. Over the past one to two decades, sandy ecosystems have been overgrown everywhere, which leads to a reduction in characteristic biotopes and a decrease in the number of reptile species living here. In our research, we began not only studying the dynamics of the population characteristics of the toad-headed agama, but also studying the succession processes taking place on the territory of the Sarykum dune massif.

Poster presentation

Timing of parental breeding influences pollution sensitivity in the European common frog (*Rana temporaria*)

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Climate change is expected to increase mean temperatures and the frequency of extreme weather events, that can lead to earlier/extended breeding seasons in temperate taxa. As a consequence, many organisms that show climate-induced phenological shifts might be exposed to environmental conditions they are not well adapted to while breeding, and their ability to cope with stressful conditions might be influenced. Here, we investigated how parental breeding time shapes the sensitivity to nitrate exposure at three consecutive life stages (embryonic, larval, juvenile) in the European common frog (*Rana temporaria*). We compared hatching success and life-stage specific survival, growth, standard metabolic rate, body condition, and acute thermal sensitivity of offspring from an earlier-breeding parental cohort (early cohort) vs. a later-breeding parental cohort (late cohort) exposed to a range of environmentally relevant concentrations of nitrate (0-100mg/L). We also investigated whether nitrate exposure experienced during the embryonic and larval stages affects physiological performance in later life stages (after metamorphosis). Our study reveals that parental breeding time affects the sensitivity to nitrate pollution at three consecutive life stages in *R. temporaria*. Breeding later in spring reduced hatching size and survival at high nitrate exposure, but also induced compensatory growth of the offspring. In both early and late cohorts, exposure to nitrate pollution reduced developmental rate and led to larger, but older larvae at the onset of metamorphosis with a greater sensitivity to warmer environmental temperatures. Standard metabolic rate, on the contrary, was neither affected by parental breeding time nor by exposure to nitrate. Exposure to nitrate pollution during embryonic and larval development led to carry-over in juvenile froglets as their sensitivity to temperature was higher. In a world highly impacted by humans, it is thus essential to give stressors a more holistic approach in order to better predict their consequences on species subjected to them.

Poster presentation

Ecological niche modelling for color morphs in Sand lizards (*Lacerta agilis*)

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Color polymorphism refers to the presence of two or more genetically inherited color phenotypes in a same breeding population. Color has an impact on organism behavior and survival, and can serve as warning signals, sex-specific recognition, enhances thermoregulations, or communicates about the behavioral state of the particular organism. While sexual selection is frequently used to explain the persistence of polymorphisms, the role of environmental factors has received little attention. Sand lizards (*Lacerta agilis*, Linnaeus 1758) are small lacertid lizards, sexually dimorphic, with sympatric color morphs found throughout temperate Palearctic. The sand lizard distribution is one of the largest ranges of reptiles' species, and it could help to understand how reptiles persist in ecosystems with anthropogenic influences. We hypothesized that different color morphs of sand lizards are distributed throughout the Palearctic realm depending on different environmental conditions. The goal of this study was to see how much morph composition in a color polymorphic lizard is explained by geographical and climate variation. We used publicly available data on sand lizard occurrence in Global Biodiversity Information Facility and environmental variables from WORLDCLIM and SEDAC databases. We categorized the sand lizard photos to ten distinct color morphs resulting in 1256 data points after thinning. We predicted the color morph ecological niches using maximum entropy models. We identified a striking link between environmental conditions and color morph distribution, implying that environmental selection acts differently on color morphs, most likely in conjunction with sexual selection.

Poster presentation

Using skeletochronology to understand how climate shapes aging and growth trajectories in amphibians

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Amphibians are particularly vulnerable to changes in their environment (e.g., precipitation and temperature regimes, habitat loss, alien species), and currently the most imperiled group of terrestrial vertebrates. In this context, understanding their life trajectories in relation to climate is of paramount importance in predicting population responses across broad geographic scales and supporting management actions. Skeletochronology (i.e., age assessment based on growth patterns observed in the bone tissue) is a widely-used quantitative instrument for studying aging and growth in vertebrates. We used Phylogenetic Generalized Linear Models to explore the aging and growth trajectory of amphibians in relation to climate, by focusing on three representative parameters extracted from published skeletochronology studies: age at sexual maturity (age_min), von Bertalanffy's growth coefficient (k), and potential reproductive lifespan (PRL). We selected the temperature and precipitation-related predictors from the WorldClim dataset of 19 bioclimatic variables. We only considered papers that: (i) computed the parameters for each sex, (ii) had a minimum sample size of ten, (iii) the location could be georeferenced, and (iv) the samples were collected between 1970-2000 (+/- 5years), to match the climate dataset from WorldClim. We obtained data for 46 species across five continents. Age at sexual maturity and PRL were significantly higher in urodelans compared to anurans. Higher values of the mean diurnal range led to higher growth in males, while age_min and PRL were predicted by the mean temperature of the wettest quarter; thus, the hotter and wetter the environment, the lower age_min and PRL, for both sexes. Age at sexual maturity was significantly lower with higher values of precipitation seasonality and lower values of annual precipitation, only in females. Our study shows which temperature and precipitation variables significantly shape the aging and growth trajectories of amphibians, and reveals the existence of sex-specific responses to these factors.

Poster presentation

Do differential activity patterns help maintain colour polymorphism in vipers from warmer environments?

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Animal colorations represent adaptations to different biotic or abiotic environmental factors and play crucial roles in predator avoidance (via crypsis, aposematism, or mimicry), inter – and intraspecific communication and sexual selection. In ectothermic animals, coloration may also be important for thermoregulation. Colour polymorphism (i.e. the occurrence of two or more phenotypic morphs in the same population) is present along numerous animal lineages, and melanism is probably the most studied type. In several Eurasian viper species of the genus *Vipera*, populations greatly vary with regards to the frequency of melanistic individuals, and the maintenance of polymorphism have been attributed to either adaptive or non-adaptive processes. Current theory would predict that melanistic vipers should be more frequent in colder environments (normally higher latitudes or altitudes), and this is mostly confirmed for the Asp Viper (*Vipera aspis*). Here we tested the hypothesis that differential habitat use and activity patterns could explain the maintenance of colour polymorphism in a viper population from warmer environment. In accordance with the thermal melanism hypothesis, we would expect melanistic vipers to predominantly use less open habitats and/or be more active at cooler temperatures, as they should be thermoregulatory superior to patterned (zigzag) individuals. Overall, our results show a weak support for the differential habitat use hypothesis, with only one habitat characteristic (microhabitat exposure) being significantly associated with morph, but only for females ($\chi^2=13.475$, $df = 6$, $p = 0.036$). However, observational data does suggest that activity patterns do differ, with melanistic vipers being especially active during overcast and rainy periods, although no differences were observed between basking site temperatures. Other adaptive as well as non-adaptive hypotheses require testing before we can gain a deeper understanding of maintenance of melanism in vipers from warmer environments.

Poster presentation

Population parameters and underlying environmental factors in the southernmost population of *Triturus cristatus* (Laurenti, 1768)

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The Northern Crested newt (*Triturus cristatus*) is the most widespread newt species, occurring from the British Isles, across parts of Scandinavia and Russia, south to the Balkan Peninsula. Three closely situated ponds in Parshevitsa, Bulgaria, present its southernmost locality, and we investigated the environmental parameters that determine its population parameters.

Monthly visits were conducted between April-November 2021. A total of 413 adults, 210 subadults and 629 larvae were registered across the ponds. There was a statistically significant difference between the ponds in terms of abundance and age classes, with Pond 1 having the lowest number of adults and both larvae and subadults were absent, Pond 2 having the highest number of larvae, and Pond 3 – the highest number of adults and subadults. Sex ratio was approximately 1:1 in all three ponds. A total of eight abiotic and five biotic factors were tested in order to determine their possible effect. There were differences in two abiotic (size of the pond and exposition) and four biotic factors (aquatic vegetation, prey diversity, fish presence and accessibility to horses). Pond 1 was the only one with fish present and had lowest food diversity, Pond 2 was the only one with no horse presence and had the highest vegetation abundance, and Pond 3 was the largest. Exposition determined longest sunlight duration for Pond 3, and shortest – for Pond 2.

The differences in abundance and age classes are likely due to the presence of fish (introduced crucian carps) and horses (free-range horses, which overgrazed and over-trample the surrounding area, causing erosion of the banks, as well as entered in the ponds and caused deterioration of the water quality and vegetation). We conclude that fish and horse presence gradually deteriorate pond quality, as evidenced by population parameters, vegetation abundance and food diversity. The size and exposition of Pond 3 likely alleviate these negative effects - still, additional measures and revision of eco-schemes for free-range horses are needed for the conservation of this protected species.

Poster presentation

Hermann's tortoise body temperatures and the thermal environment; a field study using null models

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Field observations have been made on the body temperatures of Hermann's tortoise (*Testudo hermanni boettgeri* MOJSISOVICS 1889) from the end of August to mid-October 2021 at Danilovgrad municipality, Montenegro. As a heliotherm *T. hermanni* can be expected to shuttle between hot and cool areas of the environment to regulate body temperature. To test this notion two approaches were used. Firstly, estimates of the distribution of possible body temperatures available for the tortoises were made by placing water filled copper cylinders, as null models, in various areas in their habitat; in sunny areas where the highest temperatures were expected in shaded and partially shaded areas where lower temperatures were expected. These thermal data were compared with the body temperatures of the real tortoises during daily activity (N=104). In a thermoregulatory efficiency test we compared both the field tortoise body temperatures and temperatures of the null models with the set point range of body temperatures found in a laboratory heat gradient for *T. hermanni*. The latter are the body temperatures the tortoises selected in a 'cost free thermal environment' when they were not dependent on weather, food availability or presence of predators. The extent of differences between the temperatures of field tortoise body temperatures and non-thermoregulating null models with the body temperatures *T. hermanni* selected in a thermal gradient represent a cost of thermoregulation. Our preliminary results indicate *T. hermanni* was around 70% effective as a thermoregulator during a combination of sunny, cloudy and partial weather conditions.

Session: Populations in anthropogenically modified environments



Hyla arborea

Oral presentation

The urban green toad: history, population structure and ecological adaptations of an inner-city population in Central Europe

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Worldwide urban areas have greatly expanded over the last century and are projected to continue to further increase. Amphibians are especially susceptible to these changes. The European green toad (*Bufo viridis*) seems to be a special case, since in Central Europe it is often found in urban environments. Here, we present a case study from an inner-city green toad population from Vienna (Austria).

Through historic records we traced the change of the habitat of Vienna's green toads from a former wild river system to the current fragmented urban environment.

To estimate demographic parameters as well as spatial mobility and morphometric traits of the current population we conducted a capture-recapture study from 2015 to 2021 and collected photographs, capture locations and measurements of body length and mass. We identified individuals using a semi-automated pattern-recognition program (IBEIS).

From 2015 to 2018 population size estimates steadily increased from ca. 100 to over 200 individuals and have been stable since. Annual apparent survival was usually under 0.4. Mean home range size was roughly 650 m², half the size known from natural habitats. We did not observe any movements between the main sampling site and neighboring ponds at 500 m distance.

We describe how the urban environment influences green toad population dynamics and life history and discuss potential adaptations (i.e. decreased mobility) to the inner-city habitat. Furthermore, we aim to identify habitat traits which help green toads persist in urban environments and give advice on how city-planners can support conservation of this species.

Populations in anthropogenically modified environments

Oral presentation

Site occupancy monitoring and influence of landscape features on the occurrence of the European treefrog (*Hyla arborea*) in the Upper Rhine valley

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Like most amphibians in the highly anthropized landscapes along the Rhine River, the European treefrog (*Hyla arborea*) is presumed to considerably suffer from habitat destruction and fragmentation. In order to track changes in the distribution and abundance of the species, a monitoring program using call survey has been initiated on the French side of the Rhine in 2019, and carried out again in 2021. A site occupancy model has been implemented to compare the two years, which showed the importance of the proximity with other occupied ponds and the presence of aquatic habitats overall in the prospected area. The jump in detection probability (0.72 to 0.83) and occupation rate (0.49 to 0.56) between the two years is further discussed based on the available covariables.

In order to refine our understanding of broadscale landscape features on our monitoring, more than 200 ponds have been sampled on both sides of the Rhine River, looking for reproduction clues (egg clutches and tadpoles). A multiscale analysis has then been carried out on ten landscape variables. Results suggest that a dense network of suitable breeding habitats (ponds present within a 2 km radius) is necessary for the persistence of the species. Streams and rivers conservation also seems to be of utter importance to preserve corridors between reproduction sites. These findings should be taken into account by conservation policies in the Upper Rhine valley to reverse the trend of a century of decline of the European treefrog in the region.

Oral presentation

Pesticides in the eggshells and nests of *Testudo hermanni* in a complex habitat system

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Hermann's tortoise (*Testudo hermanni*) is a chelonian species widespread in lowland and hilly parts of southeastern Serbia, where it inhabits complex habitat systems consisted of natural and human altered habitats. One such place is situated near the city of Niš, on the southwestern slopes of the Kunovica plateau. There, Hermann's tortoises inhabit degraded primarily oak forest (*Quercetum farnetto-cerris*), intersected with active and abandoned vineyards, orchards and gardens. Females lay eggs in both natural and human altered habitat patches, in late spring and in the mid-summer. In actively maintained human altered habitat patches pesticides are regularly used during the planting and growing season. In May and July 2020, within an area of 20 ha, we collected eggshells and soil samples from 10 broken tortoise nests for the purpose of the pilot study focused on accumulation of pesticides. The samples were tested for the presence of 36 pesticides most commonly used in viticulture, but only six pesticides were detected. In the soil samples we detected five pesticides: Carbofuran, Lindane, Chlordane, Bifenthrin and Cypermethrin. The sampled tortoises' eggshells contained three pesticides: Carbofuran, Cypermethrin and Deltamethrin. In only three sampling spots both eggshells and soil contained the same pesticide (Cypermethrin), while in another three sampling spots pesticides were not detected in either eggshells or soil samples. In the remaining four sampling spots, egg shells and corresponding soil samples contained different pesticides. Previous study on the same population have shown that movement range size of the female tortoises could vary between 0.04 ha and 90.07 ha, what indicates that some females could absorb pesticides far away from their nesting sites. Additionally, some soil samples which contained pesticides were collected at places which have been abandoned agricultural habitat patches for more than 30 years.

Oral presentation

Field hedgerows and wood edges are critical habitats for amphibians and reptiles in agricultural landscapes: a case study in western France

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Habitat loss is one of the main drivers of species decline around the world. In western Europe, intensification of agricultural practices led to a decline of seminatural linear habitats (hedgerows and forest edges) that are suitable for many organisms. The aim of our study was to investigate how changes in agricultural practices over time affected the distribution of amphibians and reptiles in contrasted agricultural landscapes (intensive vs. extensive) in western France. First, we described the pattern of change in hedgerow density over the past 70 years. Then we assessed how seminatural linear habitats influenced current distribution of amphibians and reptiles. Finally, we considered scenarios of future species richness distribution according to practices. Our results indicate a sharp loss in hedgerows in the last 70 years, with 39% less hedges in 2020 than in 1950. However, this decline was not homogeneous across the study area but differed according to type of soil and steepness. Among an assemblage of 22 amphibians and reptiles, a GAM modelling approach indicated that more than half were positively influenced by hedgerow and forest edges density, confirming the importance of such seminatural linear habitats for these species. Projections of hedges restoration within the study area will be used to infer future scenarios of species distribution. These results will be useful for prioritizing restoration and conservation actions and draw recommendations for management plans of hedgerow habitats.

Poster presentation

The drastic decline of *Pelophylax lessonae* in urban and rural habitats in western Poland

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Two water frog species: the pool frog *Pelophylax lessonae* (L) and the marsh frog *P. ridibundus* (R) occur sympatrically in Central Europe, and form mixed populations (genetic systems) with their hybrid, the edible frog *P. esculentus* (E). The aim of the study was to assess the species composition of water frogs in urban and rural populations and compare our current findings with the results of previous studies. The study took place in the same ponds that were surveyed by Professor Leszek Berger in 1962-1970 (Poznań, urban landscape) and 1977-1997 (Dezydery Chłapowski Landscape Park, rural landscape). Because some ponds studied in the past were destroyed or dried-up, we explored also all the other ones situated in the adjacent areas. We captured the frogs during breeding seasons 2020 and 2021 and identified them by the nuclear marker gene SAI-1. We found three types of systems in the urban area: R-E, E-E, R-E-L, and four in the rural area: R-E, L-E, E-E, R-E-L. In comparison to the historical data, we found a drastic decrease in the frequency of *P. lessonae* in both urban and rural landscapes: from 89% and 68% to 2.7% and 1.7%, respectively. At the same time, the frequency of *P. ridibundus* increased from 2.2% and 0.03% to 40% and 28.5%, respectively. A similar pattern was found for *P. esculentus*, and its frequency increased from 8.7% and 31.9% to 57.3% and 69.7%, respectively. The patterns found in both types of landscapes are in line with the current situation of both parental species in Europe. Such dynamic changes show the need for long-term monitoring of population compositions of water frogs, what is crucial for their conservation management.

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Poster presentation

***Podarcis muralis* in Vojvodina, Serbia, the spatial niche of an “urban citizen” in a highly anthropogenically modified region**

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Urbanized areas are an important feature of European landscapes and home to numerous wildlife species, whose existence sometimes depends on these areas. In this study, we assessed the factors influencing the distribution of *Podarcis muralis* in Vojvodina region. Majority of the landscape in this region was converted to agricultural and urbanized areas with related infrastructure (embankments of canals, roads, and railroads). There is very little of preserved natural habitats left. This study aims to contribute to reevaluation of ecological and conservational status of this species in urban and suburban ecosystems of this region, where it represents a constant faunistic element. To do so, we constructed ecological niche models (ENM) for this region based on more than 300 species occurrence findings and a set of 41 orographic, climatic, land-cover, and water-regime ecogeographic variables. Two modeling approaches were used: ENFA and MaxEnt. Based on model results we created habitat suitability maps that visualized ecological space and the factors affecting species' distribution. Our results suggest that the ecological determinants of the species' spatial niche in Vojvodina are defined almost exclusively by habitat land-cover variables, especially those describing urban fabric: distance from urbanized areas, frequency, and edge length of urban areas. The species shows a preference towards close proximity and high frequency of urban and suburban areas, and edges of urbanized areas, but clearly avoids areas with a high frequency of agricultural habitats. These results are in line with the biology of this species. We conclude that the common wall lizard successfully adapted to the distinctive environmental and habitat conditions in Vojvodina with a clear pattern in its spatial niche characteristics which prompt the reevaluation of common agricultural and urban maintenance practices.

Poster presentation

The urban and peri-urban herpetofauna from the city of Pitești, southern Romania

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Identifying urban and peri-urban areas that are inhabited by species of reptiles and amphibians represents the first step in understanding the way herpetofauna can adapt to anthropogenic factors. This is particularly true for regions where sampling biases have left significant gaps in our knowledge of fauna distribution, such as Romania. The aim of the presentation is to show my preliminary observations on the herpetofauna and its use of habitats in the urban and peri-urban environments in the city of Pitești, Argeș County, located in southern Romania. I identified nine species of amphibians (*Salamandra salamandra*, *Lissotriton vulgaris*, *Triturus cristatus*, *Bombina variegata*, *Bufo bufo*, *Bufotes viridis*, *Hyla orientalis*, *Pelophylax ridibundus* and *Rana dalmatina*) and nine species of reptiles (*Emys orbicularis*, *Trachemys scripta*, *Lacerta agilis*, *Lacerta viridis*, *Podarcis muralis*, *Anguis colchica*, *Coronella austriaca*, *Natrix natrix* and *Natrix tessellata*). The most abundant species in urban environments were *Podarcis muralis* and *Bufotes viridis*, with other species naturally present in the same habitats being *Lacerta viridis*, *Hyla orientalis* and *Bufo bufo*. In an artificial urban pond, there were present *Emys orbicularis* and *Trachemys scripta* (together with other exotic turtles belonging to other genera) as a result of translocation and releases. When taking into account the peri-urban areas as well, the most abundant species overall in the study area were *Bombina variegata*, *Rana dalmatina* and *Bufotes viridis*, the first two being limited to the woodland area. The most widespread species were *Hyla orientalis*, *Bufotes viridis*, *Lacerta viridis*, *Podarcis muralis* and *Natrix natrix*. These widespread species were present throughout the whole study area. I have also recorded some species of reptiles with very few observations in the Argeș County: *Natrix tessellata* and *Trachemys scripta*. Finally, I identified anthropogenic factors that negatively impact the herpetofauna, such as habitat loss, direct persecution, road mortality, invasive species and poaching.

Poster presentation

Importance of agricultural habitats for the distribution of the European fire-bellied toad across Bulgaria – implications and perspectives

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The European fire-bellied toad, *Bombina bombina*, is distributed in lowlands across Central and Eastern Europe, inhabiting puddles, shallow ponds and marshes. The species is included in the Habitats Directive of the EU, with Bulgaria on the southern edge of its distribution. Due to its preference for shallow water, it can often be found in human-created habitats, such as ditches, watering holes, etc. Although in recent years there are efforts to better understand the effects of land use on amphibians and reptiles, data is still insufficient, and for Bulgaria – virtually lacking. In an effort to better understand the importance of human-influenced habitats for *B. bombina*, we combined the latest freely available landuse data layers (from the Ministry of Agriculture) with all available point registration records for the species (from the citizen science platform SmartBirds). Using the “Intersect” function of ArcGis v.10.4, we extracted the landuse type for all records and counted the number of points for each type. In total, toads were registered in 14 landuse types. We divided these into three groups based on their origin – Natural land (NL), Natural water (NW) and Artificial habitats (AH). Results demonstrated that toads were evenly distributed across all three groups, with 277 points (36%) falling within NW, 262 (34%) – within NL, and 235 (30%) – within AH. Regarding landuse type, most records were located in Wetlands (NW, 198, 26%), Forest areas (NL, 18%) and Irrigation facilities (AH, 96, 12%), followed by Rivers (NW, 72, 9%), Grasslands (NL, 68, 9%) and Arable land (AH, 61, 8%). These results are significant in terms of the species vulnerability to agricultural practices, especially those that preserve the land use but can deteriorate habitat quality, and consequently greatly reduce *B. bombina* distribution not only in Bulgaria, but also in other parts of its range.

Poster presentation

Historical and new amphibian and reptile findings in the town of Smederevo – what has changed?

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The town of Smederevo is of considerable historical significance for Serbian batrachology and herpetology. Three amphibian species were, for Serbia, first time detected there – Danube crested newt (*Triturus dobrogicus*), Balkan spadefoot toad (*Pelobates balcanicus*) and Pool frog (*Pelophylax lessonae*). One of the rare lowland occurrences of Adder (*Vipera berus*) was also mentioned there. Still, during the second half of the XX century, Smederevo became an important industrial hub and went through considerable urbanization and habitat degradation. Surface waters were especially severely affected, with changed water regimes (parts of the waterbed of the Jezava River were filled in) and pollution by sewage and industrial waste. Consequently, some of the amphibians and reptiles published for Smederevo are, by now, present only as old (pre-1993) literature records. That is the case of the Danube crested newt, Fire salamander (*Salamandra salamandra*), Balkan spadefoot toad and Adder, the latter being declared locally extinct. The three amphibian species could still be expected in the remaining habitat fragments – since being very secretive, they could be hard to detect. Still, in the literature, some were denoted as "common". Fire salamander was reported from the habitats in the center of town. Those habitats have since been obliterated by construction. On the other hand, human activities had led to the introduction of some alien species – Kotschy's gecko (*Mediodactylus kotschyi*) and Slider turtle (*Trachemys scripta ssp.*). Also, the most recent data revealed the presence of two previously unrecorded species for Smederevo – a citizen's observation of the Smooth snake (*Coronella austriaca*) and the author's own finding of the Common spadefoot toad (*Pelobates fuscus*). Confirmation of some other potentially present species could be expected in the future, with the increased field effort.

Populations in anthropogenically modified environments

Poster presentation

Examining reptiles distribution in agricultural landscape in Cyprus

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The role of agricultural areas as niche providers for reptiles but also their negative impacts following various agricultural practices (e.g. pesticides, intensive ploughing, landscape homogenization), has long been reported. This paper is the first effort to assess the relationship between the distribution of agricultural crop types and herpetofauna of Cyprus.

For achieving this, we have compiled distributional data for all reptile species on the island combining various local databases, citizen science sources and grey literature. We then used this data to calculate species occurrence and species richness in 1km² grid cells. Using CORINE Land Cover 2018 we have reclassified agricultural land-cover types in five crop categories (irrigated crops, dry crops, woody crops, pastures, and agroforestry crops) and quantified crop extent in 1 km² grid cells. We used Shannon index of diversity (vegan package in R) as an indication of crop heterogeneity. The effect of crop categories on species richness was assessed with Generalized Additive Models (GAM).

In this study we have (i) quantified the proportion of crops extent in 1km² grid, (ii) investigated the occurrence of reptiles in various crop categories and (iii) explore how different crop categories affect herpetofauna distribution and diversity.

The outcome of this effort is an invaluable tool towards the management of agricultural areas as an important prerequisites for herpetofauna conservation in Cyprus.

Session: Reproductive systems and life histories



Zootoca vivipara

Reproductive systems and life histories

Oral presentation

Elevation influences reproductive traits and maternal conditions in an alpine gecko

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Environmental conditions and maternal effects can have profound effects on offspring phenotypes in terrestrial ectotherms. In reptiles, only a few studies examined the effects of elevation and maternal body size on reproductive traits. In general, in lizards, clutch size increases with female body size. Geckos, however, have fixed clutch sizes (one or two eggs) and thus may exhibit different patterns than those observed in other lizards. We assessed the effects of elevation and maternal body size on reproductive traits (i.e., egg mass, incubation time, size at hatching, and offspring body condition) in a adapted squamates, the Atlas day gecko, *Quedenfeldtia trachyblepharus*. We also provided new information on the reproduction biology of the Atlas day gecko (i.e., clutch size and hatching success). Atlas day geckos produced invariant clutches of one egg (n = 56). In low and high elevation populations, larger females produced heavier eggs ($r^2=0.27$) and hatchlings ($r^2=0.39$). Females from the low elevation population were in poorer body condition ($t_{52}=3.57$; $p < 0.001$), produced lighter eggs ($t_{50} = 2.78$, $p < 0.01$), and hatchlings in poorer body condition ($t_{15} = 2.63$, $p < 0.05$) than those from the high elevation population. Higher metabolic costs likely occurs in females from low land population exposed to warmer conditions.

Reproductive systems and life histories

Oral presentation

Parental care and paradox decisions: does relatedness play a role in nursery choice?

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One strategy to survive times of low nutritional availability is cannibalism, which increases survival chances of the aggressor. Cannibalism entails various costs, such as the possibility of injury during opponent subjugation and the reduction of inclusive fitness by consuming close relatives. Cannibals consistently minimise costs by being both larger and distantly related to their opponent. In species with elaborate parental care, bringing offspring close to a potential cannibal would be counter-intuitive, unless the cannibal and its potential prey are related. Male dyeing poison frogs transport their freshly-hatched tadpoles to small water pools which are often occupied by larger, potentially cannibalistic conspecifics. Here, we aim to better disentangle the apparently counter-intuitive deposition decisions by fathers: are selected pools minimising the threat of cannibalism? We investigated how the average body size of tadpoles in a pool changes in function of the density therein and their degree of relatedness. We would expect that individuals within a pool with multiple tadpoles are more similar in size and closer in relatedness. Our findings thus far support our hypothesis, suggesting that a homogenisation in body sizes in pools with higher numbers of tadpoles could be a mechanism by which the risk of cannibalism is reduced. Ongoing relatedness analysis will reveal whether this is due to the degree of relatedness or a consequence of tadpole density and therefore limited resources. However, our findings will shed light on the evolution of the seemingly paradoxical deposition behaviour of caring parents that put their offspring at risk of cannibalism.

Oral presentation

Ecotoxicology in a complex world: combined effects of pesticides and pathogens during early life in an anuran amphibian, *Rana dalmatina*

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Anthropogenic pollution can lead to a loss of biodiversity by causing mass-mortalities, but sub-lethal concentrations can also exert severe, often covert malign effects, which may be aggravated by other stress factors, such as infectious diseases. Some pesticides are known to weaken the immune function of amphibians, while others may activate their immune system or even directly damage pathogens. However, experimental studies on the interactive effects of pesticides and pathogens on amphibians have remained extremely scarce. We performed an experiment in outdoor mesocosms where we reared larvae of agile frogs (*Rana dalmatina*) exposed to environmentally relevant concentrations of one of six pesticides (two insecticides: cypermethrin, fenoxycarb; two herbicides: terbuthylazine, pendimethalin; two fungicides: tebuconazole, copper hydroxide). We measured survival, time to and mass at metamorphosis to assess direct effects of pesticides on life-history traits of amphibians. To investigate effects of pesticides on the immune defence of amphibians, we experimentally infected juveniles emerging from mesocosms with one of two pathogens (the chytrid *Batrachochytrium dendrobatidis* or a *Ranavirus*) and ten days later we noted survival, measured body mass and preserved animals for subsequent determination of infection loads using qPCR. By subjecting tadpoles to various concentrations of the same pesticides for a short period of four days and evaluating effects on mortality rates in a classic laboratory-based experiment, we also assessed to what extent simple ecotoxicological tests may help predict non-trivial malign effects of pesticides in amphibians.

Reproductive systems and life histories

Oral presentation

Preliminary data on the seasonal growth rate of the Italian cave salamander *Speleomantes italicus*

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The species growth rate is an important feature that helps us to better comprehend population dynamics, individuals' age and species reproductive potential. Species showing phenology characterized by alternating activity periods may not show constant growth, and they probably have peaks of growth occurring between their foraging seasons. Here I show my preliminary results on the seasonal growth rate in the Italian cave salamander *Speleomantes italicus*. I analysed the capture-mark-recapture data collected from six caves located in Tuscany over a year (May 2020 – April 2021). Individuals' recognition was performed using both Visual Implant Elastomers and the dorsal pattern. I assessed the seasonal growth of both the snout-vent length (SVL) and the total length (TL) operating the difference between the latest and the former individual's relative measurement. Measures of both SVL and TL were extrapolated from images in which salamanders were photographed (from above) along with a reference ruler. I built two Generalized Linear Mixed Models (one for SVL and one for TL) using the salamanders' growth as the dependent variable, while the seasons' pair (i.e., two consecutive seasons), the salamander age class and the interaction between them were considered independent factors. Both individual and population identity were used as random factors. I did not find any significant effect on both SVL and TL seasonal growth (P always ≥ 0.38). Most of the data analysed here (> 90%) were related to seasonal pairs including summer (i.e., spring-summer or summer-autumn), a very harsh period in which plethodontids most likely use energy to increase their survival rather than to increase their size. However, a further bias might have contributed to masking salamanders' seasonal growth. During each capture, salamanders underwent stomach flushing, a practice that reduced the individuals' energy accumulation. This might have affected salamanders' growth rate, which probably did not occur as it should.

Oral presentation

Drivers of sexual dimorphism variation across populations of the Italian wall lizard at different spatial scales

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The evolution of sexual dimorphism (SD) is driven by intricate interplays among multiple processes, including sexual and natural selection. Sexually selected traits should be strongly dependent on individual's body condition (condition dependent SD), which is influenced by the environment that individuals experience. As a consequence, the degree of SD may also depend on resource availability (context dependent SD). Here we analyzed condition and context dependent SD in the Italian wall lizard, *Podarcis siculus*. We studied the potential drivers of SD degree at two morphometric traits: body size (snout-vent length) and head shape (head geometric morphometrics), across ten islands and islets of the Aeolian archipelago (southern Italy). We tested three potential predictors of SD: individual body condition, ecosystem productivity and soil temperature, at within (linear mixed-effect models) and among population (linear models) scales. We found strong geographical variation of SD among islands that was unrelated to geographic distance among islands. Body condition and ecosystem productivity were the main drivers of body size SD variation, and body condition was also the main driver for head shape SD. Soil temperature played a minor role on SD expression compared to body condition and ecosystem productivity. In conclusion, males in better body condition can allocate more resources to sexually selected traits, which involve large costs, therefore, better lizard condition might promote increased degree of SD expression. Moreover, ecosystem productivity strongly determines the amount of available resources which may relax natural selection; this lead to increased selection for sexual traits and hence affect SD expression. Our results highlight that the expression of SD in the Italian wall lizard is both condition and context dependent. These results are consistent at within- and among-population scales highlighting that spatial multi-scale method represents an effective approach to understand patterns of SD expression.

Oral presentation

Variation in body size and sexual size dimorphism in the lizard *Zootoca vivipara*: The effects of reproductive mode revisited

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The transition from oviparity to viviparity has frequently occurred during the evolution of squamate reptiles. Several models of life-history theory predict that the evolution of viviparity is accompanied by increased female size, and thus higher sexual size dimorphism (SSD). Yet the corresponding empirical evidence is overall weak and inconsistent. The Common lizard (*Zootoca vivipara*), which occupies a major part of Northern Eurasia and includes four viviparous and two non-sister oviparous lineages, represents an excellent model for testing these predictions. Using body length data for nearly 14,000 individuals from 96 geographically distinct populations, which cover the almost entire species' range and include all six lineages, we analysed how sex-specific adult body size and SSD is associated with reproductive mode, lineage identity, and seasonality (the strongest predictor of geographic body size variation in previous studies on this species).

Variation in male size was weak and poorly explained by our predictors. In contrast, female size and SSD varied considerably; the effect of reproductive mode was highly significant and exceeded that of lineage identity and seasonality. We hypothesize that the marked effect of reproductive mode in this study is due to an effective control for multiple confounding factors, including the very recent origin of viviparity (and partly oviparity!) in this study system, the weak and irregular variation in male size, as well as an appropriate covering of geographic and genetic diversity of the species. This study is supported by the German Research Foundation (DFG, grant RO 4168/1-3).

Oral presentation

Microplastics have sublethal effects on amphibian larvae and lead to post-metamorphic carry-over effects: A study with polyethylene microplastics and *Xenopus laevis*

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Microplastics (MPs) are a fast-growing source of environmental pollution which is of increasing concern as MPs are abundant, ubiquitous, and persistent over time and might represent potential risks for wildlife and ecosystems. However, such risks are still mostly unknown for amphibians. If ingested with the natural food source, MPs act as artificial fibers that reduce food quality with possible ramifications for growth and development. We investigated the effects of MPs ingestion (polyethylene, particle size: 34-50 µm) in two life stages (i.e., larvae and juveniles) of the African clawed frog (*Xenopus laevis*). We assessed effects of MPs ingestion on larval growth, development, gut length, body condition as well as their standard metabolic rate (SMR). We conducted morphometric measurements (fore- and hindlimb length, body width) in juveniles to determine possible carry-over effects of MPs ingestion across metamorphosis. In both life stages, we investigated whether MPs accumulated in the body. We found that MPs ingestion resulted in sublethal effects on development and metabolism in larval *X. laevis*, lead to carry-over effects on juvenile body shape, and accumulated in the animal body. In larval stage, gut length increased in response to MPs ingestion indicating that MPs fibers induced digestive plasticity. Body mass and body condition were similar across experimental groups, indicating that larvae fully compensated for low nutrient and energy density by developing longer intestines. However, SMR and age increased and decreased in response to MPs ingestion, respectively. In juveniles, body width was larger in animals exposed to MPs during larval stage. Our findings provide first insights into the complex effects of MPs on amphibians across life stages, and suggest that juvenile amphibians might be a major transfer path for MPs from freshwater to terrestrial ecosystems. Future experiments need to consider field prevalence and abundance of various MPs in amphibians in order to allow for generalizations and the development of suitable conservation actions.

Oral presentation

Optimal body mass-length ratio during hibernation for *Emys orbicularis* (Linnaeus, 1758) – European Pond Turtle

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The aim of this study was to determine optimal body mass/length of carapace ratios of *Emys orbicularis* before hibernation. We obtained 213 measurements of wild-caught and captive-bred (81 wild-caught and 14 captive-bred individuals from wild-caught parents) turtles during 9 years (2011-2019) and determined regression curves that show intervals for optimal or suboptimal body mass/length ratios before winter dormancy. Using this data we designed an online calculator (accessible at <https://emyscalc.github.io/>) that facilitates the process of establishing if a turtle has an optimal body mass/length ratio before hibernation. We also gathered 45 measurements at the end of the hibernation period which showed an average body mass loss of 5.32%. The present study offers practical means of assessing if an *Emys orbicularis* individual is fit for hibernation.

Poster presentation

Ontogenetic aspects of sexual dimorphism in the Caspian Whip Snakes (*Dolichophis caspius*)

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Sexual dimorphism in body size has been observed in several snake species. Natural and sexual selections and their interaction lead to differences in morphological traits between males and females. During the ontogeny, various changes in body parts can occur between the sexes. Different body sizes can reflect different behaviours and reduce interpopulation competition for resources. We examined sexual dimorphism in Caspian Whip Snake (*Dolichophis caspius*). We analysed the suburban population in Belgrade (Serbia). The sample consists of 72 females and 66 males of three age categories (juveniles < 40 cm of SVL, subadults from 40.1 to 70 cm of SVL and adults > 70.1 cm of SVL). We analysed eight morphometric parameters (standard body length - SVL, tail length - TL, jaw length - JL, cranial length - CL, postparial length - PPS, head width - HW, mouth length - ML and mouth width - MW) and body weight - BM. Descriptive statistics showed that the values of all analysed traits were higher in adult males (the mean values of total body length were 137 and 117 cm for adult males and females, respectively), and ANOVA showed that these differences were statistically significant. The same analyses showed significant sexual dimorphism in the relationship between body length and tail length (SVL/TL). After correction for body size (ANCOVA with SVL as a covariate), sexual dimorphism was not expressed in any of the analyzed traits. In juvenile and subadult individuals, sexual dimorphism is not pronounced. The observed differences in morphological traits may be related to distinct features of life history, so our further research will consider the differences between the sexes in the growth rate, the size at which they reach sexual maturity, diet, predation, and activity patterns.

Reproductive systems and life histories

Poster presentation

Developmental stages of *Triturus ivanbureschi*

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Staging tables based on the external morphological characteristics are important for the needs of comparative embryological studies as they represent a baseline. Large-bodied newts (*Triturus* spp.) have a transparent mucoid capsule that enables insight into the external morphology of the embryo during development. *Triturus* newts are characterized by the presence of chromosome 1 syndrome where about 50% of the offspring stops developing and dies during the mid embryonic period on the tail bud stage. In this study, we focused on the normal development of Balkan crested newt (*Triturus ivanbureschi*). During the breeding season animals were kept in the yard of the institute, in plastic tubs (from 200 to 400 liters), covered with a mosquito net that allowed natural day-night regime. Plastic strips were provided as an imitation of underwater vegetation, on which the females laid their eggs. Eggs were collected every morning and moved to Petri dishes at the controlled experimental conditions. The embryos were kept in the laboratory at a temperature of 20°C. Photographs and time-lapse recordings of developing embryos were taken every day at the same time. Compared to already published staging tables for newts and based on the appearance of certain morphological structures, such as gills, extremities, balancers, etc., we distinguished 37 different stages of normal embryonic development of *T. ivanbureschi*. Still, differences in external morphology due to the balanced lethal chromosome 1 syndrome need further research.

Poster presentation

Meiotic chromosomes, synaptonemal complexes (SC) in the cryptic forms of the Euroasian complex *Zootoca vivipara* with multiple sex chromosomes

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Among lacertid species, *Zootoca vivipara* is a rare case possessing both simple and multiple sex chromosomes (male $2n=36$, Z1Z1Z2Z2/ Z1Z2W female $2n=35$). Some changing in W sex chromosome (morphology, cytogenetic structure) accompany active form-formation and subspeciation in the *Z. vivipara* complex. Alterations in some characters of sex chromosomes, in particular multiple sex chromosomes, may influence the process of meiosis and may play a role in isolation and speciation. Therefore, early spermatogenesis and oogenesis of the western cryptic form (reorganized W sex chromosome) have been firstly examined. The data obtained have been compared with previous studies of Safronova, Kupriyanova and colleagues on the males and females of eastern (Russian) cryptic form as well as with studies on males of *Takydromus* species – a closely related to *Zootoca* genus. The correlation in the process of meiosis of both forms of *Zootoca* were observed: in males ($2n=36$), all 18 synaptonemal complexes' (SCs) bivalents, including SCs Z1Z1Z2Z2 sex chromosomes, appeared to be fully synapsed; moreover, their regular segregation with forming of haploid spermatocytes, 18 chromosomes at the metaphase 1 stage, were constantly revealed. However, the mitotic and meiotic characteristics of the *Takydromus* species differed.

In eastern (Russian) cryptic form of *Z. vivipara* ($2n=35$), unlike males, 19 SC elements at the pachytene – diplotene prophase1 stage of female meiosis were identified (16 SCs fully synapsed autobivalents and 3 SCs elements may be a sex chromosomes' univalents). The lampbrush chromosomes have been observed in the eastern and the western cryptic forms, but, in the later, their exact number is still unknown. However, at the telophase-anaphase stages, some disturbances in the segregation of chromosomes have been revealed. Finally, some complex and even ambiguous behavior of sex chromosomes in female meiosis of *Z. vivipara*, related to the problems of female meiotic drive and subspeciation, need future studies

Reproductive systems and life histories

Poster presentation

Sublethal effects of two pyrethroid insecticides, commonly used in agriculture and mosquito control, on agile frog larvae

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Pyrethroid insecticides are widely used, because they are considered as less toxic to mammals and birds than organochlorine and organophosphate insecticides. However, previous studies showed that they are highly toxic to aquatic organisms, including amphibians. Although usually these pesticides are applied to agricultural and household insect pests, and veterinary applications, pyrethroids can enter surface water bodies via spray drift, spills, or direct application for mosquito control.

In our study we investigated the effects of two pyrethroids, deltamethrin (DM) and etofenprox (ETF) in environmentally relevant concentrations on a native amphibian species, the agile frog (*Rana dalmatina*). We exposed tadpoles to one of two concentrations (0.003 µg/L or 0.03 µg/L) of the insecticide or a solvent control (0.03 µl/L ethanol) throughout the whole larval development. We measured tadpole survival, growth, development and behavior.

We found that neither of the chemicals affected survival, body mass at metamorphosis or activity at either concentrations. However, exposure to the higher concentration of DM or ETF significantly delayed metamorphosis. Our results suggest that exposure to environmentally relevant concentrations of these chemicals can negatively affect the natural populations of agile frog, since temporary water bodies, where these animals often develop, can dry out rapidly, which can lead to the loss of the whole annual brood.

Reproductive systems and life histories

Poster presentation

Captive breeding, embryonic and larval development of the zimmermann's poison-frog *Ranitomeya variabilis* (Zimmermann and Zimmermann, 1988), (Anura: Dendrobatidae)

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Following recent taxonomic changes in *Ranitomeya variabilis* (Zimmermann and Zimmermann, 1988), we provide herein, for captive bred specimens from French Guiana a description of husbandry in captivity; image-based measurements of total length (TOL) and surface area of individuals throughout embryonic to larval development using the SAISAQ (Semi-Automatic Image based Surface Area Quantification) tool. Furthermore, we provide a detailed larval staging from early staged embryo to metamorph. Clutches of *R. variabilis* were composed of four to six dark grey eggs, deposited in a mass and wrapped in a colorless gelatinous capsule. Deposition occurs mainly on the largest and basal axils of phytotelma, and less often in photographic film containers, offered as artificial phytotelma. The obtained growth data by SAISAQ showed in comparison with SAISAQ data of other *Ranitomeya* species a similar growth pattern. However, we noted a remarkable TOL difference between *R. variabilis* and other *Ranitomeya* tadpoles, which was also traceable in the ontogenetic data. The developmental stages largely corroborate with the generalized Gosner's staging system for anuran embryos and larvae. Development from the stage eight of Gosner through metamorphosis took 80 to 91 days, with a survival rate of 54%. Differences were observed mainly on the labia and teeth differentiation, that occurred a stage later than expected in the general staging system. As well as, the atrophy of the oral apparatus, which started one stage earlier than expected in two tadpoles. The provided data brings new knowledge about reproductive biology of this species and supports in situ breeding programs for conservation purposes. We are confident that these data can support the identification of *R. variabilis* tadpoles in natural environment and the differentiation of tadpoles from the sister species *R. amazonica*. As well as, providing new data, that can help to clarify the biogeographic distribution and taxonomic arrangement of the species.

Poster presentation

Life history changes observed over 17 years in a Common Toad population from Serbia

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One long-term studied local population of *Bufo bufo* situated in the outskirts of southeastern Belgrade (Serbia) was assessed for changes in body size and age over a 17 years period, at different moments: A (2005: n=47), and B (2013-2014: n=74) for females, and A (2005: n=94), B (2013-2014: n=151) and C (2020-2021; n=302) for males. The snout-vent length of the females did not change over time (Mann Whitney U: $Z=-0.89$; $p>0.05$), but their body mass was higher in moment B (Mann Whitney U: $Z=-4.7$; $p<0.05$). The snout-vent length of the males increased over the study period (Kruskal-Wallis: $\text{Chi-sq}=26.82$; $p<0.05$), but their body mass remained similar (Kruskal-Wallis: $\text{Chi-sq}=26.82$; $p>0.05$). We observed a sexual dimorphism, females being larger than males ($\text{SD}=1.31$, Mann Whitney U: $Z=-16.08$; $p<0.05$). The median age increased over time in both females (moment A - 4 years old; moment B - 5 years old; Mann Whitney U: $Z=-6.97$; $p<0.05$), and males (moment A - 4 years old, B - 5 years old, C - 5 years old, Kruskal-Wallis: $\text{Chi-sq}=40.68$; $p<0.05$). Age of sexual maturity (i.e., minimum age observed) remained similar over the study period in both females (3 years old) and males (2 years old). Longevity (i.e., maximum age observed) increased over the study period, from 6 to 7 years old in females, and from 6 to 9 years old in males. We revealed positive changes in both body size and age of the studied population over a 17-years period of monitoring. Thus, our study provides a baseline that will help monitor and quantify the impact of habitat changes that were only recently observed in the area (i.e., deforestation, since 2019).

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