



XX European Congress  
of Herpetology  
Milan, 2-6 September 2019

# PROGRAM & ABSTRACTS



UNIVERSITÀ  
DI PAVIA



**mnm** Museo di Storia  
Naturale di Milano

**Organising Institutions:**

Societas Europaea Herpetologica ([www.sch-herpetology.org](http://www.sch-herpetology.org))

Università degli Studi di Milano ([www.unimi.it](http://www.unimi.it))

Università degli Studi di Pavia ([www.unipv.it](http://www.unipv.it))

Museo di Storia Naturale di Milano (<https://www.comune.milano.it/museostorianaturale/>)

Università degli Studi di Milano-Bicocca ([www.unimib.it](http://www.unimib.it))

**Local Organising Committee:**

Gentile Francesco Ficetola, Roberto Sacchi, Stefano Scali, Raoul Manenti, Edoardo Razzetti, Emilio Padoa-Schioppa, Chiara Trabella, Marco Mangiacotti, Andrea Melotto, Mattia Falaschi, Alan Coladonato

**Scientific Committee:**

Gentile Francesco Ficetola, Roberto Sacchi, Stefano Scali, Raoul Manenti, Alan Coladonato, Andrea Melotto, Mattia Falaschi, Edoardo Razzetti, Marco Mangiacotti, Stefano Canessa, Andrea Villa, Claudia Corti, Isolde van Riemsdijk, Ben Wielstra, Jan-Dieter Ludwigs, Gianpaolo Montinaro, Jelka Crnobrnja-Isailović, Mathieu Denoël, Antigoni Kaliontzopoulou, Dan Cogalniceanu, Judit Vörös, Petros Lymberakis, Marco Zuffi, Marco Sannolo

**Secretariat:**

Martina Muraro, Elisabetta Gozzo

**Herp photos:**

Mattia Falaschi, G. Francesco Ficetola, Andrea Melotto, Daisy Pensotti

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## *Welcome to Milan!*

We are glad to host the XX European Congress of Herpetology, which has been co-organized by the University of Milan, the University of Pavia, the Natural History Museum of Milan and the University of Milano-Bicocca. Participants from 40 countries across the world registered for the event, and we hope that our congress will be a great moment of scientific discussions and social exchanges. We will have about 200 talks and more than 100 posters covering a very wide range of topics, from systematics to conservation issues, from methodological tools to ecology and ethology. We also have a rich program of social events that will certainly facilitate the exchanges and the discussions.

Milan is also a beautiful city with a great nightlife, and can be an excellent starting point for visiting the rich biodiversity of Italy. We really hope you will enjoy the congress and your stay in Milan!

The Organizing Committee

## *Our logo*

The “Biscione” (big snake) in the act of consuming a child is the heraldic emblem of Milan since the Middle Ages. According to the legend Bonifacio, lord of Pavia, was married with Bianca, daughter of the Duke of Milano. While Bonifacio fights against the Saracens, his son is kidnapped and devoured by a giant snake. When coming back from the war, Bonifacio searches the snake, kills him, and finds his son still alive!

In our logo, the Biscione is eating a herpetologist, instead of the Bonifacio’s son...



# General Information

E-mail: [seh-congress@unipv.it](mailto:seh-congress@unipv.it)

Website: <http://seh-congress-2019.unipv.it/>

## **Venue - Scientific activities:**

University of Milan, Via Celeste Clericetti 2-16, Milan. See Pag. 332-333 for maps.

## **Opening hours**

2 September, Monday 9:00–18.00

3 September, Tuesday 8:30–18.30

4 September, Wednesday 8:30–18.30

5 September, Thursday 8:30–18.30

6 September, Friday 8:30–17.00

## **Internet access**

Free Wi-Fi is available in the University halls.

## **Coffee**

Coffee, tea, soft drinks, mineral water and small cakes will be served during the coffee breaks in the area adjacent to the Aula.

*Please note that it is prohibited to consume meals or drinks in the Aula.*

## **Lunches**

*Please note that lunches are not included in the registration fee.* However, lunches can be bought in the canteen at the 2<sup>nd</sup> floor of the building (the price for a full meal is 6,27 EUR).

## Social Events

### **Opening Ceremony and Welcome Party**

Museo Civico di Storia Naturale di Milano, Corso Venezia 55, Milano

*Date:* 2 September (Monday)

*Time:* 18:30 hrs

Admission is free for congress participants and for their registered accompanying persons.

The museum can be easily reached from the congress venue via underground (Line 1; stop: Palestro). See Pag. 333 for the map. To go to the welcome party, we will move from the congress venue around 18.00.

*Meeting point:* Entrance of the Aula

### **Congress photo**

A picture of participants will be taken and later uploaded to the congress website.

*Place:* stairs of the congress building

*Time:* 4 September, 14:30

### **Gala dinner**

*Date:* 5 September, between 19:00 and 23:00

*Location:* Palazzo Cusani – Salone Umberto I

The location of the gala dinner can be easily reached via underground (Line 2, Lanza). To go to the gala dinner, we will move from the congress venue around 18.30. *Meeting point:* Entrance of the Aula

*Admission:* Only with ticket, tickets are available in limited number at the registration desk

*Price:* 50 EUR

## Field Excursions

### **1. Visit to the subterranean laboratory for the study of *Speleomantes salamanders* [Stazione Biospeleologica di Besolagno (Savignone, GE)]**

*Date:* 7 September (Saturday)

*Services provided:* Transportation by bus, English speaking guide, lunch

*Meeting point:* University of Milan (Via Celoria)

*Departure time:* 9:00

*Arrival to Lambrate metro and railway station (Milan):* 17:00

*Costs:* 45 €

### **2. Field trip in the Ticino Regional Park – La Fagiana (Magenta – Milano), to look for reptiles and amphibians**

*Date:* 7 September (Saturday)

*Services provided:* Transportation by bus, English speaking guide, lunch

*Meeting point:* University of Milan (Via Celoria)

*Departure time:* 9:00

*Arrival to Lambrate metro and railway station (Milan):* 17:30

*Costs:* 55 €

### **3. Field trip for Amphibians and Reptiles of Orobie Prealps [Passo San Marco, Mezzoldo (BG)]**

*Date:* 7 September (Saturday)

*Services provided:* Transportation by bus, English speaking guide, lunch

*Meeting point:* University of Milan (Via Celoria)

*Departure time:* 8:15

*Arrival to Lambrate metro and railway station (Milan):* 17:30

*Costs:* 50 €

**More informations about the field trips are available on our website, <http://seh-congress-2019.unipv.it/field-trip/>**

## Presentation Guidelines

### **Poster display**

Posters will be on display during the whole Congress. Authors are responsible for setting up their poster at the beginning of the congress and removing it after the end of the congress.

*Venue:* Numbered poster boards are in the Congress building, ground floor and gallery

*Poster size:* The maximum size allowed for each poster is 70 cm wide and 100 cm high.

*Poster position:* Vertical. Posters that do not adhere to the dimensional limits cannot be displayed.

*Set-up:* 2 September 11:00–18:00

*No poster should be set up during the Opening Ceremony!*

*Poster removal:* 6 September 14:00–17:00

*No poster should be removed during the Closing Ceremony!*

*Poster session* 4 September 14:45–18:00

Drinks and snacks will be served nearby the Aula.

### **Oral presentations**

The length of the oral presentations will be 12 minutes with 3 minutes discussion, 15 minutes altogether. Please contact the technical staff if you have additional requests.

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# Schedule overview

<b>Mon, 2nd</b>			
10:00-18:00	Registration (congress venue, V. Clericetti 2)		
18:30-21:00	Welcome party (Museum of Natural History, Corso Venezia 55)		
<b>Tue, 3rd</b>			
	(congress venue, V. Clericetti 2)		
	Aula Levi	Room 501	Room 502
9:00-9:30	Opening		
9:30-10:15	Plenary (Uller)		
10:15-10:45	Coffee break		
10:45-12:30	Ecology and Ethology	Conservation	Biogeography & Distributions
12:30-14:00	Lunch break		
14:00-16:00	Ecology and Ethology	Conservation	Morphology & Physiology
16:00-16:30	Coffee break		
16:30-18:00	Ecology and Ethology	Conservation	Morphology & Physiology
<b>Wed, 4th</b>			
9:00-9:45	Plenary (Schmidt)		
9:45-10:30	Evolution & Phylogeny	Response plan to Bsal	Herps in islands
10:30-11:00	Coffee break		
11:00-12:30	Evolution & Phylogeny	Response plan to Bsal	Herps in islands
12:30-14:00	Lunch break		
14:00-14:30	SEH is 40! (Böhme)		
14:45-18:00	Poster session		
<b>Thu, 5th</b>			
9:00-9:45	Plenary (Crottini)		
9:45-10:30	Evolution & Phylogeny	Agricultural landscapes	Redlists
10:30-11:00	Coffee break		
11:00-12:30	Evolution & Phylogeny	Agricultural landscapes	Redlists
12:30-14:00	Lunch break		
14:00-16:00	Evolution & Phylogeny	Methods	Redlists
16:00-16:30	Coffee break		
16:30-18:30	SEH general meeting		
19:30-23:00	Social Dinner (Via Brera)		
<b>Fri, 6th</b>			
9:00-9:45	Plenary (Measey)		
9:45-10:30	Ecology and Ethology	Hybrid zones	Osteology
10:30-11:00	Coffee break		
11:00-12:30	Ecology and Ethology	Hybrid zones	Osteology
12:30-14:00	Lunch break		
14:00-15:15	Ecology and Ethology	Hybrid zones	Osteology
15:15-16:00	Coffee break		
16:00-17:00	Closing and awards ceremony		

# Detailed program

Tuesday, 3rd			
	Aula Levi	Room 501	Room 502
9:00-9:30	Opening ceremony		
9:30-10:15	<b>Plenary:</b> T. Uller Evolutionary origin and spread of a sexually selected phenotype		
10:15-10:45	Coffee break		
	<b>Ecology and Ethology</b>	<b>Conservation</b>	<b>Biogeography &amp; Distributions</b>
10:45-11:00	M. D. Barquero et al. Effect of temperature on the visual displays of the Jacky Dragon	F. Andreone et al. A conservation strategy for the amphibians of Madagascar: old and new threats in an iconic biodiversity-rich country	D. Chamorro et al. Forecasting distributions and competitive interactions for European vipers
11:00-11:15	V. Bjelica et al. Turning, tugging, running and reversing: survival strategies of the European Pond Turtle	F. Belluardo et al. Characterization of the herpetological diversity of the Andringitra Massif (South-East Madagascar)	U. Enriquez-Urzelai et al. Does geography rule over biology? Lessons from the diversification of green lizards
11:15-11:30	G. Blouin-Demers & J. E. Paterson Density-dependent habitat selection predicts fitness and abundance in a small lizard	G. Deso et al. The Tyrrhenian painted frog ( <i>Discoglossus sardus tschudi</i> ) in the Port-Cros National Park (France): definition of a monitoring protocol, past and present distributions, potential threats and management measures	P. Ginal et al. Ecophysiology predicts the fundamental niche of native and invasive populations of the African clawed frog ( <i>Xenopus laevis</i> )
11:30-11:45	M. A. Carretero et al. Infection of parthenogenetic lizards by blood parasites does not support the "red queen hypothesis" but reveals the costs of sex	A. Kozakova et al. Cost-effective analysis of mitigation measures for amphibian protection on roads	Y. V. Kornilev et al. Updated distribution and ecology of the Alpine newt ( <i>Ichthyosaura alpestris</i> , Laurenti, 1768) (Amphibia: Salamandridae) in Bulgaria
11:45-12:00	V. Clement et al. Climatic preferences of a sand lizard population ( <i>Lacerta agilis linnaeus</i> 1758) in W Germany	M. Ferreira et al. Incorporating evolutionary history into conservation planning – the case of Madagascar's mantellids	M. Solé et al. Ignored for centuries: why is our knowledge on bromeliad frogs from Brazil so poor?
12:00-12:15	U. Dajčman et al. Does sympatry influence the parasite prevalence and parasite load in two competing lacertids?	J. Foster et al. Progress and challenges in restoring a nationally extinct species: <i>Pelophylax lessonae</i> in the UK	
12:15-12:30	M. Denoël & H. Cayuela Conservation ecology of crested newts: High site infidelity in a network of small ponds	D. Guinart et al. An endemic newt ( <i>Calotriton arnoldi</i> ) as a tool for conservation of riverside habitat	
12:30-14:00	Lunch break		
	<b>Ecology and Ethology</b>	<b>Conservation</b>	<b>Morphology &amp; Physiology</b>
14:00-14:15	C. Dittrich & M.-O. Rödel You cant't always get what you want – female mate choice in the European common frog	J. Hall et al. Wind farms – a neglected opportunity for viper conservation?	I. Avella et al. Evolving trends in snake venom research: a review of the last 60 years of publications
14:15-14:30	C. Ducotterd et al. Metabarcoding as a tool to determine feeding behaviour – is the European pond turtle a threat for other endangered species?	A.M.Hantzschmann & U. Sinsch Focussing on yellow-bellied toad ( <i>B. variegata</i> ) populations in the Westerwald region – consequences for regional species conservation management	S. Baškiera & L. Gvoždík Repeatability but not heritability of metabolic rate in the Alpine newt

14:30-14:45	A. Gini et al. First observation of convergent character displacement in a syntopic community of two Italian lizards: <i>Podarcis muralis</i> and <i>P. siculus</i>	J. Herder & R. Van Leeningen Data from long term toad patrols show moderate decline of common toad ( <i>Bufo bufo</i> ) in the Netherlands	L. B. Mali et al. Chronology of limb regeneration in captive born juvenile of the European blind cave salamander
14:45-15:00	O. J. Glavaš et al. Influence of copper on frogs' early development	J. Kovarik et al. Herpetofauna and road construction – complex mitigation measures	C. Dursun et al. High or low? A test of the relations between altitude and keratinized spines
15:00-15:15	M. Glogoški et al. Differences in behaviour of two lacertids in competitive relationship: <i>P. siculus</i> and <i>P. melisellensis</i>	A. Krása New locality of <i>Zamenis longissimus</i> in the Czech Republic	L. Coppari et al. Quantitative and structural variation in skin secreted toxins across populations of the frog <i>Bombina pachypus</i> (Anura: Bombinatoridae)
15:15-15:30	G. Gollmann & B. Gollmann Twenty-four years, fourteen thousand toads: An interim report of a population study on <i>Bombina variegata</i>	D. O'Brien et al. Constructed urban wetlands can support healthy amphibian populations but do they bring people into contact with nature?	M. Hlubeň et al. Evolution and causes of variability in evaporative water loss in eyelid geckos (Eublepharidae)
15:30-15:45	A. Golubović et al. Oscar deserving performance: death-feigning in dice snakes	K. Poboljšaj Amphibians & roads - in pursuit of the common objectives	N. T. Kolarov et al. Patterns of correlations and locomotor specialization in anuran limbs: association with phylogeny and ecology
15:45-16:00	J. Kloskowski et al. Costly avoidance: anuran spatial antipredator responses against fish at the oviposition and larval stages	M. Pabijan et al. Decline in amphibian abundance and biomass in Niepolomice Forest (S Poland) over a 50 year period	F. C. Origgi et al. Ranid herpesvirus 3 and Bufonid herpesvirus 1, two new viral agents associated with proliferative skin disease in free ranging common frogs ( <i>Rana temporaria</i> ) and common toads ( <i>Bufo bufo</i> ) in Europe
16:00-16:30	Coffee break		
16:30-16:45	T. Koppetsch et al. To stick or not to stick: functional ecomorphology of limbs and tail and their significance for locomotion in three species of eublepharid geckos (Squamata: Sauria: Eublepharidae)	R. Rozinek et al. Lowering <i>Bd</i> prevalence as a part of mitigation measures	R. A. Regnet et al. Population structure and ventral polychromatism of <i>Helicops infrataeniatus</i> Jan, 1865 (Serpentes, Dipsadidae) in subtropical Brazil
16:45-17:00	B. Lejeune et al. Progenesis as an intrinsic factor of ecological opportunity in newts	M. Van Schingen et al. Herpetofauna conservation in the light of international trade: the perspective of governmental conservationists	K. Ruthsatz et al. Energetic efficiency of metamorphosis in <i>Rana temporaria</i>
17:00-17:15	E. Lunghi et al. Can the Eltonian niche be predicted? A test with sardinian cave salamanders	A. Spitzen – Van Der Sluijs & Tariq Stark How (and why) to detect infectious diseases?	N. Serén et al. How (will) ectotherms cope with changing environments? A test with a lizard under contrasting ecological pressures
17:15-17:30	E. Mizsei & A. Móri Predation on the endangered hungarian meadow viper ( <i>Vipera ursinii rakosiensis</i> ) by badger ( <i>Meles meles</i> ) and fox ( <i>Vulpes vulpes</i> )	M. Szabolcs et al. Balkan herps project: spatial conservation priorities for the amphibians and reptiles in the Balkan peninsula	A. S'Khifa et al. Ecophysiological traits of lacertidae lizards in extreme environments in Morocco
17:30-17:45	L. M. Schulte & F. Bossuyt Sex-pheromones in neotropical frogs: communication beyond calling	J.-P. Vacher et al. Conservation genetics of <i>Bufo viridis</i> (Anura: Bufonidae) in the upper Rhine Valley	R. Smolinský et al. Color in focus - can the sand lizard still surprise us?
17:45-18:00	E. Twomey et al. Mechanisms for color convergence in a mimetic radiation of poison dart frogs	D. Villero et al. Optimizing the creation of new wild populations of the Montseny brook newt ( <i>Calotriton arnoldi</i> )	A. Urošević et al. Morphological integration of the cranium and axial skeleton in European newts

Wednesday, 4th			
	Aula Levi	Room 501	Room 502
9:00-9:45	<b>Plenary:</b> B. R. Schmidt Can we halt the decline of amphibians?		
	<b>Evolution &amp; Phylogeny</b>	<b>Response plan to Bsal</b>	<b>Herps in islands</b>
9:45-10:00	B. Antunes et al. Evolutionary consequences of isolation by environment in two ecomorphologically distinct salamanders, <i>Salamandra salamandra bejarae</i> and <i>S. s. almanzoris</i>	<b>F. Pasmans &amp; A. Martel</b> Setting the scene: the Bsal threat to European urodeles	V. Avramo et al. Island effect on intraspecific and intersexual morphological variability in <i>Podarcis siculus</i>
10:00-10:15	S. Carranza et al. Systematics and diversification of the geckos of the genus <i>Pristurus</i>	<b>W. Beukema et al.</b> Beneficial amphibian thermal behaviour remains constrained by the environment in the face of pathogenic invasion	S. Baeckens et al. Dalmatian oddities: functional divergence of head shape in lizards on two peculiar islands in the Adriatic
10:15-10:30	U. Dajčman et al. Conservation genetics of the leopard tortoise <i>Stigmochelys pardalis</i>	<b>K. Preissler et al.</b> Saving the fire salamander: ex situ breeding of insurance populations in the face of an expanding pathogen	C. Donihue et al. Trait changes in <i>Podarcis erhardii</i> from a replicated island introduction experiment in Greece
10:30-11:00	Coffee break		
11:00-11:15	C. Dufresnes et al. Speciation with hybridization: the phylogeography of green toads ( <i>Bufo</i> ) revisited	<b>A. Spitzen &amp; M. Gilbert</b> <i>Batrachochytrium salamandrivorans</i> in the netherlands: past, present and future perspectives	M. D. Scherz & C. Hawlitschek The forgotten hotspot: a decade of research into the herpetofauna of the Comoros archipelago
11:15-11:30	N. Feiner Evolutionary lability in <i>hox</i> cluster structure and gene expression in <i>Anolis</i> lizards	<b>S. Lötters et al.</b> Germany, the hotspot of Bsal emergence	A. Herrel et al. Lizards on islands as models for rapid adaptive diversification
11:30-11:45	F. Martínez-Freiria et al. Biogeographic history of <i>Cerastes</i> vipers. A tale from the world's warmest deserts	<b>D. Herczeg et al.</b> Emerging infectious diseases of amphibians in Hungary	A. V. Mohan et al. Population genetics of the Andaman day gecko ( <i>Phelsuma andamanensis</i> ; blyth, 1861) on the Andaman islands; an idiosyncratic species of unique biogeographic origins but limited genetic structure
11:45-12:00	A. Kaliontzopoulou et al. Are <i>Podarcis</i> wall lizards an adaptive radiation? Joining phylogenetic and phenotypic evidence	<b>D. Lastra González et al.</b> Recent findings of the amphibian killer fungus <i>Batrachochytrium salamandrivorans</i> , the spreading goes on	K. C. Webster et al. Morphologically <i>diskinkt</i> : comparing island populations of skinks in the Comoros archipelago
12:00-12:15	V. A. Gorin et al. Phylogeny and biogeography of narrow-mouthed frogs of the genus <i>Microhyla</i> (Amphibia: Microhylidae)	<b>M. Vila-Escalé et al.</b> Amphibian population management in southern europe to contain and eliminate a local outbreak of <i>Batrachochytrium salamandrivorans</i> (Bsal)	
12:15-12:30		<b>S. Canessa et al.</b> Developing an early response plan to invasion by <i>Batrachochytrium salamandrivorans</i>	
12:30-14:00	Lunch break		
14:00-14:30	SEH is 40! (Böhme)		
14:45-16:00	Poster session		
16:00-16:30	Coffee break		
16:30-18:00	Poster session		

Thursday, 5 <sup>th</sup>			
	Aula Levi	Room 501	Room 502
9:00-9:45	<b>Plenary:</b> A. Crottini Evolution and conservation of the herpetofauna of Madagascar		
	<b>Evolution &amp; Phylogeny</b>	<b>Agricultural landscapes</b>	<b>Redlists</b>
9:45-10:00	V. Gomes & A. Kaliontzopoulou Evolutionary variation in morphology-performance associations in <i>Podarcis</i> wall lizards	M. E. Ortiz-Santaliestra Improving effectiveness of pesticide risk assessment for amphibians and reptiles	<b>Red List assessments</b>
10:00-10:15	K. Candan et al. Molecular phylogeny of Valentin's lizard, <i>Darevskia valentini</i> , (boettger, 1892) reveals new genetic lineages and may suggest taxonomic reconstruction	G. Montinaro & J.D Ludwigs Towards a guidance document on risk assessment for amphibians and reptiles: telemetry data of sand lizards ( <i>Lacerta agilis</i> ) to record field data relevant to registration	<b>Red List assessments</b>
10:15-10:30	F. Martínez-Freiria et al. Towards evidence-based species delimitation in Eurasian vipers	C. Leeb et al. Potential pesticide exposure during the post-breeding migration of the common toad ( <i>Bufo bufo</i> ) in a vineyard dominated landscape	<b>Red List assessments</b>
10:30-11:00	Coffee break		
11:00-11:15	G. Mazepa et al. Revision of the taxonomy and phylogenetic history of Palearctic water frogs ( <i>Pelophylax</i> )	M. A. Carretero What is known and what is still to be known about the function of lizards in agro-environments	<b>Red List assessments</b>
11:15-11:30	M. Mezzasalma et al. Identification and characterization of two transposable elements and an amniote ultra-conserved element (UCE) in the genome of <i>Zootoca vivipara</i>	V. Mingo et al. The use of plant protection products and its impact on reptile	<b>Red List assessments</b>
11:30-11:45	M. Mirč et al. Common wall lizard ( <i>Podarcis muralis</i> ) vs urbanization	M. Biaggini & C. Corti Some fields are better than others. Ecological effects of agricultural management on lizards	<b>Red List assessments</b>
11:45-12:00	N. Özdemir et al. Phylogenetic systematics of <i>Bufo bufo</i> species group in Turkey	Simbula et al: A multi-disciplinary approach to assess pesticides effects on lizards ( <i>Podarcis siculus</i> )	<b>Red List assessments</b>
12:00-12:15	C. Pizzigalli et al. Systematics revision of the <i>Mesalina olivieri</i> species complex (Squamata: Lacertidae) from North-West Africa with the description of one new species	L. Boualit et al. Effects of pesticides and antibiotics on the early development of the model amphibian organism: <i>Xenopus laevis</i>	<b>Red List assessments</b>
12:15-12:30	N. A. Poyarkov & C. Suwannapoom Narrow-mouthed frogs of the subfamily Asterophryinae (Anura: Microhylidae): an addition to Indochinese herpetofauna	Ortiz-Santaliestra " Opportunities for networking and scientific cooperation in the field of pesticide risk assessment for herpetofauna: the COST Action PERIAMAR	<b>Red List assessments</b>
12:30-14:00	Lunch break		

	<b>Evolution &amp; Phylogeny</b>	<b>Methods</b>	<b>Redlists</b>
14:00-14:15	L. Rancilhac et al. The phylogenetic relationships of the Salamandridae (Amphibia: Urodela): a history of deep and shallow reticulations	M. E. Allentoft et al. Uncovering the genomic and metagenomic potential in old ethanol-preserved snakes	Red List assessments
14:15-14:30	D. Salvi et al. Evolutionary history of the leaf-toed gecko <i>Euleptes europaea</i> reveals recent biogeographic cross-road between Sardinia and Corsica	A. Barbi et al. The role of artificial shelters in the study of alpine reptiles in the Stelvio National Park	Red List assessments
14:30-14:45	A. S. Silva & M. Wilkinson A new approach to build the amphibian tree of life	A. Costa et al. Count data for monitoring single populations: a herpetological case study and general validation	Red List assessments
14:45-15:00	E. N. Solovyeva et al. Spotted toad-headed agamas of the <i>Phrynocephalus guttatus</i> complex: in search of species boundaries	S. Giachello & E. Lunghi How to analyse coloration in amphibians and reptiles?	Red List assessments
15:00-15:15	G. Velo-Antón et al. Comparative landscape genetics reveals the evolution of viviparity reduces genetic connectivity in fire salamanders	E. Ringler et al. Functional magnetic resonance imaging as novel tool to study anuran cognition?	Red List assessments
15:15-15:30	J. Vörös et al. Landscape genomics of the Caspian whipsnake ( <i>Dolichophis caspius</i> ) across Eastern Europe and Western Asia	M. A. Samlali et al. Evaluation of a method to monitor a population of the moroccan painted frog ( <i>Discoglossus scovazzi</i> ) under extreme environmental conditions (High Atlas, Morocco)	Red List assessments
15:30-15:45	A. Yanchukov et al. Microsatellite genotypes of seven hybridogenetic unisexual species of rock lizard and their putative bisexual parents (genus <i>Darevskia</i> sp.) Suggest complex patterns of hybrid ancestry	M. Sassoè-Pognetto et al. Use of a wireless ultrasound probe as a portable, non-invasive method for studying reproductive biology in wild populations of the asp viper ( <i>Vipera aspis</i> )	Red List assessments
15:45-16:00	O. Zinenko et al. Multilocus phylogeny of small vipers (subgenus: <i>Pelias</i> ; genus: <i>Vipera</i> ) based on genome-guided ddRAD nuclear DNA markers		Red List assessments
16:00-16:30	Coffee break		
16:30-18:30	SEH general meeting		
19:30-23:00	Social Dinner (Via Brera)		



Friday, 6 <sup>th</sup>			
	Aula Levi	Room 501	Room 502
9:00-9:45	<b>Plenary:</b> J. Measy The future of our planet's amphibians and reptiles: a view from invasion science		
	<b>Ecology and Ethology</b>	<b>Hybrid zones</b>	<b>Osteology</b>
9:45-10:00	C. Rodríguez et al. Perch higher and be quieter: acoustic adaptation and sound radiation patterns in <i>Allobates femoralis</i>	W. Babik et al. Differential introgression across newt hybrid zones – evidence from replicated transects	A. M. Bauer & J. D. Daza The osteology of geckos: interesting skeletons come in small packages
10:00-10:15	M. Falaschi et al. Invasive species override habitat change in determining newt decline at the regional scale	A. Bellati et al. Alternative outcomes of hybridization in water frogs (gen. <i>Pelophylax</i> ) revealed by in vitro crossings and molecular approaches	J. D. Daza & A. M. Bauer The herpetofauna of a coastal tropical forest of Myanmar, during the mid-Cretaceous
10:15-10:30	E. Ringler et al. Clutch cannibalism by adult poison frogs	J. L. Delgado Transcriptome based snp markers reveal dynamics of the marbled newts' hybrid zone	M. Delfino et al. Global herpetological osteology: a preliminary overview on the European taxa
10:30-11:00	Coffee break		
11:00-11:15	M. Ringler et al. Compost dwellers: strategic feeding and use of indirect cues in actively foraging poison frogs	K. Dudek et al. Pervasive introgression of MHC genes in newt hybrid zones	M. Joshi and C. Koch Identification without external characters – digging deeper in the skull morphology of fossorial Leptotyphlopidae using µCT
11:15-11:30	C. Rato et al. Climate change and local adaptation: challenges of a lizard whose sex is determined by environmental temperature	C. Dufresnes et al. Hybrid zone genomics of European anurans	M. Maričić et al. Sexual dimorphism in shell shape and size of European pond turtle in Serbia
11:30-11:45	L. Roner et al. Trophic ecology of Alpine salamander, ( <i>Salamandra atra atra</i> , Laurenti, 1768)	S. Freitas et al. Deep branch gene flow in a hybridization rich diversification process	T. Matthews et al. Identifying fossil frog assemblages from southern Africa based on a review of the ilia of extant taxa
11:45-12:00	G. Rosa et al. Trophic strategy and prey availability of two populations of <i>Euproctus montanus</i> (Savi, 1838)	V. Gvozdik et al. Slow-worm lizards ( <i>Anguis</i> ) – a new reptile model in hybrid zone studies	T. Skawiński et al. Comparative cranial osteology of <i>Podarcis</i> lizards (Squamata: Lacertidae): evolutionary and palaeontological implications
12:00-12:15	S. Salvidio et al. Thirty years of cave salamander population dynamics... and beyond	I. Freitas et al. Habitat selection and morphological variation across human-altered habitats in secondary contact zones: the case of western mediterranean vipers in northern Iberia	M. Tañanda et al. New data on the Middle Jurassic lizards from Great Britain
12:15-12:30	A. J. Coladonato et al. Morph-specific pattern of aggression throughout the season in <i>Podarcis muralis</i>	I. Van Riemsdijk et al. Spatial variation in introgression along the common toad hybrid zone	A. Villa et al. Comparative skull osteology and phylogeny of the Italian vipers
12:30-14:00	Lunch break		

14:00-14:15	J. Speybroeck Flaunting males and cautious females – seasonal variation in behaviour and habitat use of the fire salamander ( <i>Salamandra salamandra</i> ) in a temperate north-western european forest	M. Ruiz-Miñano et al. Unravelling the geographical patterns of introgression in wall lizards using landscape genetics	P. Wagner The genus <i>Adolfus</i> sternfeld, 1912
14:15-14:30	L. Tomović et al. Population characteristics of the nose-horned viper ( <i>Vipera ammodytes</i> ) on Golem Grad Island (North Macedonia)	S. Steinfartz et al. The evolutionary consequences of local adaptation and hybridization in the worlds' only sea going lizard - the Galápagos marine iguana	L. C. M. Wencker et al. Difficulties in using continuous characters in specimen-level osteological phylogenetic analyses of lacertid lizards
14:30-14:45	G. Vimercati et al. Invasive subtropical toads allocate more resources to growth and maintenance over reproduction and storage in a mediterranean environment	T. Vučić et al. What <i>Triturus</i> newts can tell us about diversification and evolution within hybrid zone?	
14:45-15:00	V. Zwahlen et al. Sex-biased dispersal in the asp viper	B. Wielstra Hybrid zone movement in crested newts	
15:00-15:15	B. Barzaghi et al. How many shades of yellow? Factors determining the dorsal coloration pattern of the fire salamander ( <i>Salamandra salamandra</i> )	W. Yang & T. Uller Genomic patterns of parallel hybrid zones provide insight into the causes of introgression and speciation in common wall lizard	
15:15-16:00	Coffee break		
16:00-17:00	<i>Awards and closing ceremony</i>		





# Plenary lectures

## **EVOLUTIONARY ORIGIN AND SPREAD OF A SEXUALLY SELECTED PHENOTYPE**

TOBIAS ULLER

Department of Biology, Lund University

Evolutionary biologists aim to understand how novel characters arise and why they spread through populations. Here I will review our ongoing research on the evolution of a striking suite of exaggerated traits – morphology, coloration and behaviour – in common wall lizards (*Podarcis muralis*). Genomic analyses reveal that the suite of traits, traditionally associated with the ‘*nigriventris*’ phenotype, originated recently close to modern Rome. The phenotype has since spread and eventually introgressed into a distantly related lineage in western Italy. I will explain why the traits are favoured, what limits their spread, and how this creates a mosaic of phenotypic and genetic variation of the species in Italy.

## **CAN WE HALT THE DECLINE OF AMPHIBIANS?**

BENEDIKT R. SCHMIDT

Info fauna karch, Neuchatel, Switzerland & Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

The global decline of amphibians is one of the best-known examples of the global loss of biodiversity. I will first provide a short description of the magnitude of amphibian population declines, including those in protected areas, and I will then briefly talk about the reasons for the declines. The main part of the talk will be about solutions: what kind of research is necessary to halt and reverse amphibian declines? I will argue that a shift in conservation research is necessary. More knowledge on the causes will not necessarily lead to better amphibian conservation. Instead, we should and can do research that directly informs amphibian conservation.

## **EVOLUTION AND CONSERVATION OF THE HERPETOFAUNA OF MADAGASCAR**

ANGELICA CROTTINI

CIBIO/InBio, Centro de Investigação em Biodiversidade e Recursos Genéticos  
Universidade do Porto, Rua Padre Armando Quintas,7  
4485-661 Vairão, Portugal

Madagascar hosts an almost unparalleled concentration of endemic, diverse and endangered flora and fauna. Despite having four centuries of biological explorations, undescribed diversity is still widespread and is occurring at both poorly explored and in better-studied areas, and field exploration is still playing a crucial role in new species discoveries. The product of our field-based research resulted in the identification and description of several new species, in proposing new classifications, and is currently contributing to uncover the pattern of species diversification of multiple radiations of amphibians and reptiles. The native amphibian fauna of Madagascar is constituted by five independent radiations with 100% species-level endemism. Among these, the mantellid frogs is the most diversified family of anuran that here underwent a wide species radiation that resulted in the evolution of a plethora of morphological, ecological and reproductive traits. We combined the use of evolutionary genomics with the collection of life history traits and the use of comparative phylogenetic methods to investigate the evolutionary association between life-history and morphological traits and test for their contribution to the diversification of this group.

Although Madagascar has a well-developed network of protected areas, ongoing habitat destruction, political instability, emergent infectious diseases and invasive species are weakening protection in many reserves and put others at risk, threatening Madagascan unique biodiversity. It is in view of all this that we invested in the establishment of a solid network of researchers and initiatives that is aiming at protecting the unique herpetofauna of Madagascar.

**THE FUTURE OF OUR PLANET'S AMPHIBIANS AND REPTILES:  
A VIEW FROM INVASION SCIENCE**

JOHN MEASEY

Stellenbosch University, Stellenbosch, South Africa

In 2000 years, the future of our planet could have one of four potential outcomes: end of humanity, Pangea, use some/preserve others, and conservation earth. While we have already started along the trajectory toward Pangea, our actions in the near future will determine whether we can achieve a future where we retain areas that are preserved and herpetologically distinct. Among the global change drivers, invasion science is increasing in importance because of a continuing and near exponential increase in trade. We need all herpetologists to help generate the data needed to prevent the Floridarisation of herpetology. In this presentation, I outline a number of ways in which herpetologists can push back against herpetological Pangea: (i) characterising current invasive species, (ii) determining the breadth of invasion debt, and (iii) scoping the traits of all species to establish a rational basis for future trading. Although it was only conceived in the 1950s, invasion science now draws on almost all disciplines in biology, including phylogenetics, population biology, evolutionary ecology and modelling, to name but a few. However, our best chances of preventing herpetological Pangea may come from social science studies of what drives the trade in reptiles and amphibians. The challenge for us is to retain the potential for inspiring reptile and amphibian pets for coming generations, without peppering our environment with propagules of invasive species.

**ROOTS OF THE EUROPEAN HERPETOLOGICAL SOCIETY: FOUR DECADES OF THE „SOCIETAS EUROPAEA HERPETOLOGICA“ (SEH)**

WOLFGANG BÖHME

Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Adenauerallee 160. D-53113 Bonn, Germany

The first idea to create a supranational European herpetological society was independently born in the late 1970-ties, viz. in Paris and in Bonn. Herpetologists of the natural history museums of both cities, viz. Alain Dubois and myself, found it regrettable that Europe lacked a supranational scientific herpetological journal while there were three such journals in the US. This led to a first informal discussion meeting in Paris in January 1979 with nine representatives from the French side, and two from the German side. As a result a second discussion meeting with delegates of additional European countries was decided and scheduled for the summer, to be held again in France, but this time in Montpellier. Participants came, apart from France and Germany, also from Austria, Italy, The Netherlands, and Spain, but the discussion lost itself in minor questions, without clear results. Nonetheless, an invitation was made for a foundation meeting in Bonn, in September of the same year. This invitation was followed by delegates from Austria, France, Germany, Italy, The Netherlands, and Turkey, and the meeting resulted in the formal foundation of the „Societas Europaea Herpetologica“ (SEH). A first council was elected, with Josef Eiselt from the Vienna Natural History museum as the first president. The main goal of the whole activity, viz. the creation of a supranational scientific journal, was achieved with „Amphibia-Reptilia“, first published by the Academic Publishing House Wiesbaden, meanwhile by Brill in Leiden.

According to the new statutes, Ordinary General Meetings (OGMs) were held biannually in varying cities and countries including Austria, Czechoslovakia, France, Germany, Greece, Hungary, Luxemburg, The Netherlands, Poland, Portugal, Russia, Slovenia, and Turkey. Scientific committees of the society include the Conservation and the Mapping Committee which published a voluminous distribution atlas of the European. More recently, a Taxonomic Committee was founded to accompany the increasing changes and amendments in European herpetological nomenclature. The society's flagship journal „Amphibia-Reptilia“ appears in its 40th volume (with an impact factor of meanwhile around 1.3). Since 2008, as an online reference, „Herpetology Notes“ are published for shorter communications. The talk gives an outline of the society's successful development from 1979 until its current 40<sup>th</sup> anniversary.







## Session: Biogeography and distribution

**FORECASTING DISTRIBUTIONS AND COMPETITIVE INTERACTIONS FOR EUROPEAN VIPERS**

DARÍO CHAMORRO

Biogeography, Diversity and Conservation research team, Depto. de Biología Animal, Facultad de Ciencias, Universidad de Málaga, E-29071, Málaga, Spain.

ANTONIO-ROMÁN MUÑOZ

Biogeography, Diversity and Conservation research team, Depto. de Biología Animal, Facultad de Ciencias, Universidad de Málaga, E-29071, Málaga, Spain.

RAIMUNDO REAL

Biogeography, Diversity and Conservation research team, Depto. de Biología Animal, Facultad de Ciencias, Universidad de Málaga, E-29071, Málaga, Spain.

FERNANDO MARTÍNEZ-FREIRÍA

CIBIO/InBio, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto, R. Padre Armando Quintas, 4485-661, Vairão, Portugal.

European vipers (*Vipera*) show parapatric distributions as result of distinct evolutionary trajectories and specific ecological requirements. Competitive interactions among species at range margins play an important role in the sustainment of distributional patterns. Taking into account the competition between species in a heterogeneous climate along their distribution areas, three biogeographical cases are predicted to happen: 1) autoecological segregation, when a low favorable climate is stronger than biological interactions; 2) sympatric coexistence, when an optimal climate for both species provides high abundance of resources, minimizing the effect of competitive interactions; and 3) sinecological segregation, when in sub-optimal areas, one species, better adapted to this conditions, could exclude the other by competition. Fuzzy logic distribution models provide a useful framework to analyze and identify these interactions. Using a presence/absence matrix for five western vipers (*V. aspis*, *V. berus*, *V. lataste* and *V. seoanei*) and a set of climate variables at ~10x10 km resolution, we studied species favorability for current and future climate scenarios. Regions of overlap between species pairs were analyzed to identify the three biogeographical cases in both periods of time. Results emphasize the generalist character of *V. aspis*, with a predicted range largest than the currently observed, and the strong competitive character of *V. berus*, *V. latastei* and *V. seoanei* at range margins, which might be limiting the expansion of the former species. Despite a high uncertainty in our predictions, future favorable areas are restricted to coastal and mountain regions, which is translated into important changes in species interactions.

**DOES GEOGRAPHY RULE OVER BIOLOGY? LESSONS FROM THE  
DIVERSIFICATION OF GREEN LIZARDS**

URTZI ENRIQUEZ-URZELAI

CIBIO/InBIO Research Centre in Biodiversity and Genetic Resources, Vairão, Portugal

FERNANDO MARTÍNEZ-FREIRÍA

CIBIO/InBIO Research Centre in Biodiversity and Genetic Resources, Vairão, Portugal

INÊS FREITAS

CIBIO/InBIO Research Centre in Biodiversity and Genetic Resources, Vairão, Portugal

ANA PERERA

CIBIO/InBIO Research Centre in Biodiversity and Genetic Resources, Vairão, Portugal

ÍÑIGO MARTÍNEZ-SOLANO

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

DANIELE SALVI

Università degli Studi dell'Aquila, L'Aquila, Italy

ANTIGONI KALIONTZOPOULOU

CIBIO/InBIO Research Centre in Biodiversity and Genetic Resources, Vairão, Portugal

The balance between speciation and extinction (i.e. diversification), together with dispersal, dictate biodiversity patterns, which vary enormously among regions and taxa. This implies that diversification depends both on 'intrinsic' organismal traits and 'extrinsic' factors associated to specific regions. Areas such as the Mediterranean Basin which underwent intense paleogeographic changes are known to have favored isolation and promoted diversification. Non-exclusively, the acquisition of key phenotypic traits (e.g. morphological innovations or shifts in climatic tolerances to exploit new niches) could drive diversification. The contribution of intrinsic traits and extrinsic factors to diversification, however, remains elusive. Here we test the contribution of paleogeography and phenotypic evolution in the diversification of green lizards (*Timon* and *Lacerta*). We reconstructed a complete dated phylogeny including all known lineages within species. Further, we characterized each lineage using 1) ecologically relevant morphological traits from museum specimens and 2) climatic niche characteristics (thermal and hydric conditions across each lineage's distribution). Diversification analyses revealed that green lizards diversified steadily, without major rate shifts, and suggested that (paleo)geography played a significant role in this process. Body and relative head size, as well as climatic niche characteristics, exhibited significant phylogenetic signal pointing to higher dissimilarity among close relatives than expected under random processes. In contrast, thermal niche characteristics were more similar among close relatives than expected by chance. However, trait-dependent diversification analyses did not lend support to any influence of these variables on diversification rates. Our results suggest that the geographic history of the Mediterranean Basin governed the diversification of green lizards.

**ECOPHYSIOLOGY PREDICTS THE FUNDAMENTAL NICHE OF NATIVE AND INVASIVE POPULATIONS OF THE AFRICAN CLAWED FROG *Xenopus laevis***

PHILIPP GINAL

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

ANTHONY HERREL

Muséum National d'Histoire Naturelle, Paris, France

JOHN MEASEY

University of Stellenbosch, Stellenbosch, South-Africa

MOHLAMATSANE MOKHATLA

University of Stellenbosch, Stellenbosch, South-Africa

DENNIS RÖDDER

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

One main threat promoting the worldwide amphibian decline is the introduction of non-indigenous amphibians, like the African Clawed Frog *Xenopus laevis*, which is now one of the widest distributed amphibians occurring on four continents with ongoing expansion including large parts of Europe. Species Distribution Models (SDMs) and the concept of ecological niche are essential to predict the invasive risk of those species. Previous efforts to predict distributions of invasive amphibians have mainly focused on correlative approaches but these can be vulnerable to extrapolation errors when projecting species' distributions in non-native ranges. Recently, more robust process-based models, which use physiological data like critical thermal limits, or hybrid models of both approaches were used for this purpose. Previous correlative SDMs for Europe predict different patterns in the potential distribution but it is likely that these models do not access the full invasive potential. Based on physiological performance trials we calculated size and temperature depending response surfaces, which were scaled to the species' range matching the critical thermal limits. These ecophysiological performance layers were used in a standard correlative SDM framework to predict the potential distribution in South Africa and Europe. We found that thermal performance differed significantly among native and invasive populations indicating some degree of fundamental niche change, which lead to different potential distribution patterns for the native and invasive populations in the respective ranges. Our hybrid-SDMs revealed that *X. laevis* has a much higher invasive potential than previous correlative models predicted for Europe.

**UPDATED DISTRIBUTION AND ECOLOGY OF THE ALPINE NEWT *Ichthyosaura alpestris* (LAURENTI, 1768) (AMPHIBIA: SALAMANDRIDAE) IN BULGARIA**

BORISLAV Y. NAUMOV

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria; E-mail: herpetology\_bg@yahoo.com

GEORGI S. POPGEORGIEV

Bulgarian Society for the Protection of Birds & National Museum of Natural History, Sofia, Bulgaria

YURII V. KORNILEV

National Museum of Natural History, Sofia, Bulgaria

Department of Integrative Zoology, Faculty of Life Sciences, University of Vienna, Vienna, Austria

DIMITAR G. PLACHIYSKI

Bulgarian Society for the Protection of Birds & National Museum of Natural History, Sofia, Bulgaria

ANDREI J. STOJANOV

National Museum of Natural History, Sofia, Bulgaria

NIKOLAY D. TZANKOV

National Museum of Natural History, Sofia, Bulgaria

The Alpine newt (*Ichthyosaura alpestris*) is a glacial relict in Bulgaria, occurring only in some mountainous regions. Here, we present results of a long-term herpetological survey of Bulgaria, including sampling specifically for the aquatic phase of *I. alpestris*. We increased the known vertical and geographic distribution of the species – it occurs in seven mountains (in 42 10×10 km UTM squares, 22 of which previously unreported); we detected it for the first time in two mountains: Pirin and Karvav Kamak. However, several potentially suitable mountains remain under-sampled. A Maxent model with strong predictive power (test AUC = 0.988) based on 10 uncorrelated variables revealed that about 5% of the country is potentially suitable. Elevation is the best predictor in the model, followed by slope, while precipitation seasonality and the potential evapotranspiration indicate the importance of the presence of (seasonal) water bodies. *Ichthyosaura alpestris* seemingly prefers to breed in (semi-) natural shallow water bodies with muddy bottom, grassy banks, limited reed/bulrush, without fish, near/in forested habitats. The registered relative abundances (range: 0.002–1.183 ind./trap hour), the estimated population densities (0.2–7.1 ind./m<sup>2</sup>), and the sex ratios (from 0.25♂ : 1♀ to 7.29 : 1) vary widely among and within mountains.

**IGNORED FOR CENTURIES: WHY IS OUR KNOWLEDGE ON BROMELIAD  
FROGS FROM BRAZIL SO POOR?**

MIRCO SOLÉ

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil and Herpetology Section,  
Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

JUDIT VÖRÖS

Department of Zoology, Hungarian Natural History Museum, Budapest, Hungary

IURI RIBEIRO DIAS

Programa de Pós-Graduação em Zoologia, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

AMANDA SANTIAGO FERREIRA LANTYER-SILVA

Programa de Pós-graduação em Zoologia, Instituto de Biociências, Universidade Estadual Paulista Júlio de Mesquita Filho,  
Rio Claro, Brazil

AILA DA SILVA SALINAS

Programa de Pós-Graduação em Zoologia, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

EUVALDO MARCIANO-JR

Centro de Conservação e Manejo de Fauna da Caatinga, Universidade Federal do Vale do São Francisco, Petrolina, Brazil

RENAN NUNES COSTA

Programa de Pós-Graduação em Sistemas Aquáticos Tropicais, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

LEANDRO OLIVEIRA SANTOS

Departamento de Ciências Básicas, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Brazil

VICTOR GOYANNES DILL ORRICO

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

With 1.087 described amphibian species Brazil ranks first in worldwide amphibian richness. While new species are still discovered near the traditional research centers in southeastern Brazil several new species have been described from the Atlantic Forest of northeastern Brazil. Research in these areas has been traditionally performed through expeditions led by researchers from southeastern Brazil. The recent expansion of the Brazilian public University system has resulted in a much larger number of professional herpetologists studying the amphibian diversity of northeastern Brazil. Especially bromeliad frogs, often neglected by researchers who focus on amphibians found at ponds and streams, have been experiencing an increased attention. Half of the 14 known species in the genus *Phyllodytes* have been described in this millennium and at least three more species have already been identified as new by means of integrative taxonomy and are awaiting formal description. In our presentation we will draw an historical overview starting with the first species in the genus described by the Prince Maximilian of Wied-Neuwied in 1824 and reaching the present-day new species boom. We will also provide new data on tadpole morphology, bioacoustics, reproductive modes and on the ability of tadpoles of species within the genus to act as biocontrollers of mosquito larvae, thus helping to staunch the flow of mosquito borne diseases as dengue, chikungunya and zika.



## Session: Conservation



**A CONSERVATION STRATEGY FOR THE AMPHIBIANS OF MADAGASCAR:  
OLD AND NEW THREATS IN AN ICONIC BIODIVERSITY-RICH COUNTRY**

FRANCO ANDREONE

IUCN SSC Amphibian Specialist Group-Madagascar/Museo Regionale di Scienze Naturali, Via G. Giolitti 36, I-10123  
Torino, Italy

JEFF DAWSON

Durrell Wildlife Conservation Trust, Les Augrès Manor, La Profonde Rue, Trinity, Jersey JE3 5BP, Channel Islands, UK

SERGE NDRIANTSOA

IUCN SSC Amphibian Specialist Group-Madagascar, Antananarivo 101, Madagascar

ANDOLALAO RAKOTOARISON

Mention Zoologie et Biodiversité Animal, Faculté des Sciences, Domaine des Sciences et Technologies, Université  
d'Antananarivo, B.P. 906. Antananarivo 101, Madagascar

TSANTA RAKOTONANAHARY

Department of Veterinary and Sciences, Faculty of Medicine, BP 375 Antananarivo 101, Madagascar

ANGELICA CROTTINI

CIBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO, Universidade de Porto, Campus Agrário de  
Vairão, Rua Padre Armando Quinta, no 7, 4485-661, Vairão, Portugal

Madagascar hosts one of the richest amphibian communities in the World with more than 360 currently described species and at least 200 taxa already identified and waiting formal description. The native amphibian fauna of Madagascar is constituted by five independent anuran radiations. All species and the 88% of the genera are endemic to Madagascar, ranking the country third in the world for number of endemic amphibian species. Like many other frog faunae around the world, Madagascar's amphibians are severely under threat, with about the 30% of all described species listed in the threatened categories of the IUCN Red Listing. Habitat loss and fragmentation was and remain one of the main threats to amphibian conservation, although other factors are feared to worsen such situation. Namely, the discovery of the amphibian chytrid fungus and the presence of the invasive alien toad *Duttaphrynus melanostictus*. In this contribution we present the main activities carried out over the last 13 years by the IUCN SSC Amphibian Specialist Group Madagascar. This effort led to the organization of two crucial workshops (ACSAM workshops in 2006 and in 2014), leading to the development of the Sahonagasy Action Plan and, more recently, the New Sahonagasy Action Plan 2016-2020 and the recruitment of an amphibian program team in country which is following the implementation of the action plan through an open partnership approach.

**CHARACTERIZATION OF THE HERPETOLOGICAL DIVERSITY OF THE  
ANDRINGITRA MASSIF (SOUTH-EAST MADAGASCAR)**

**FRANCESCO BELLUARDO**

CIBIO Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Rua Padre Armando Quintas, Campus de Vairão, 4485-661 Vairão, Portugal

**DARWIN DÍAZ QUIRÓS**

CIBIO Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Rua Padre Armando Quintas, Campus de Vairão, 4485-661 Vairão, Portugal

**JAVIER LOBON ROVIRA**

CIBIO Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Rua Padre Armando Quintas, Campus de Vairão, 4485-661 Vairão, Portugal

**GONÇALO MIRANDA ROSA**

Institute of Zoology, Zoological Society of London, Outer Circle, Regent's Park, NW1, 4RY, London, United Kingdom  
Centre for Ecology, Evolution and Environmental Changes (CE3C), Faculdade de Ciências da Universidade de Lisboa, Edifício C2, 5º Piso, Sala 2.5.46 Campo Grande, 1749-016 Lisboa, Portugal

**MALALATIANA RASOAZANANY**

Mention Zoologie et Biodiversité Animal, Domaine des Sciences et Technologies, Université d'Antananarivo, B.P. 906. 101 Antananarivo, Madagascar

**FRANCO ANDREONE**

Museo Regionale di Scienze Naturali di Torino, Via Giovanni Giolitti 36, 10123 Torino, Italy

**ANGELICA CROTTINI**

CIBIO Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Rua Padre Armando Quintas, Campus de Vairão, 4485-661 Vairão, Portugal

Madagascar is one of the richest biodiversity hotspots, with amphibians and reptiles showing exceptional levels of diversity and endemism. A great portion of this herpetological diversity has been discovered and formally described in the last three decades, following a period of intensified fieldwork research in combination with the emergence and use of standardized molecular markers. Through the years this enabled a fast and accurate identification of species and candidate new species. Despite the advances, numerous areas are still poorly characterized. The Andringitra Massif, located in the south-east portion of the island, is one of these areas. We surveyed 10 sites across the Andringitra Massif over a period of ca. 50 days between November and December 2018 in a total sampling area of ca. 1,300 km<sup>2</sup>. To enable a fast and accurate identification of the collected material, genomic DNA was extracted from about 500 samples. A fragment of the cytochrome oxidase subunit I (COI) and a fragment of the 16s rRNA genes were amplified and sequenced for the reptiles and amphibians, respectively. Different biodiversity indexes were calculated (species richness, phylogenetic diversity, weighted endemism and phylogenetic endemism) for each surveyed site in order to characterize the biodiversity patterns of the surveyed area. This study represents the first large-scale characterization of the herpetofauna of the region of the Andringitra Massif.

**THE TYRRHENIAN PAINTED FROG *Discoglossus sardus tschudi* IN OTTH, 1837 IN THE PORT-CROS NATIONAL PARK (PORT-CROS AND LEVANT ISLANDS, VAR DEPARTMENT, FRANCE): DEFINITION OF A MONITORING PROTOCOL, PAST AND PRESENT DISTRIBUTIONS, POTENTIAL THREATS AND RECOMMENDATIONS FOR MANAGEMENT MEASURES.**

GREGORY DESO

Ahpam (Herpetological Association of Provence Alpes Méditerranée), House of associations, 384 route de Caderousse, F-84100 Orange. [ahpam.contact@gmail.com](mailto:ahpam.contact@gmail.com)

RÉMI DUGUET

Alcedo Fauna and Flora, 85 impasse Baslaval, F-07110 Sanilhac. [rduguet@expertise-ecologique.fr](mailto:rduguet@expertise-ecologique.fr)

PAULINE PRIOL

StatiPop-Consulting in animal population monitoring, 4 avenue de Nîmes, F-34190 Ganges.

[pauline\\_priol@yahoo.fr](mailto:pauline_priol@yahoo.fr)

AURELIEN BESNARD

EPHE, Biogéographie et Ecologie des Vertébrés, Centre d'Ecologie Fonctionnelle et Evolutive, UMR 5175, 1919 route de Mende, 34293 Montpellier cedex 5, France. [aurelien.besnard@cefe.cnrs.fr](mailto:aurelien.besnard@cefe.cnrs.fr)

DAVID GEOFFROY

Parc-Cros National Park, 181 Castel Sainte Claire alley, BP70220, F-83406 Hyères Cedex.

[david.geoffroy@portcros-parcnational.fr](mailto:david.geoffroy@portcros-parcnational.fr)

A presence / absence protocol was used to model the probability of detection of Tyrrhenian Painted Frog and the probability of occupation of breeding sites, mainly temporary streams or "wadis". The investigations took place in 2018 in the island of Port-Cros and in 2019 in the island of Levant. The influence of different habitat covariates could be tested. The results make it possible to define an optimized monitoring protocol for monitoring the status of Sardinian Discoglossal populations in the National Park. This study also makes it possible to compare the distribution of the population of the island of Port-Cros with the data available since the 1950s, to identify potential threat factors and to propose management measures.

**INCORPORATING EVOLUTIONARY HISTORY INTO CONSERVATION PLANNING  
– THE CASE OF MADAGASCAR’S MANTELLIDS**

MIGUEL FERREIRA

Faculty of Sciences of University of Porto / CIBIO-InBio - Research Center in Biodiversity and Genetic Resources, Porto,  
Portugal

ANGELICA CROTTINI

CIBIO-InBio - Research Center in Biodiversity and Genetic Resources, Porto, Portugal

WALTER COCCA

Faculty of Sciences of University of Porto / CIBIO-InBio - Research Center in Biodiversity and Genetic Resources, Porto,  
Portugal

FRANCESCO BELLUARDO

Faculty of Sciences of University of Porto / CIBIO-InBio - Research Center in Biodiversity and Genetic Resources, Porto,  
Portugal

SÍLVIA B. CARVALHO

CIBIO-InBio - Research Center in Biodiversity and Genetic Resources, Porto, Portugal

Protected areas are a major tool to preserve biodiversity. Several prioritization strategies have been recently developed to optimize conservation areas networks, including methods that maximize representation of long-term evolutionary history. However, these strategies are frequently unsuitable for groups that have undergone rapid radiations.

As one of the most iconic biodiversity hotspots, Madagascar hosts thousands of endemic species. Its amphibian diversity originated from post-isolation colonisations and within-island diversification, with all the native species being endemic to Madagascar. The mantellid frogs are the most diversified lineage of Malagasy amphibians and have been exposed to habitat destruction since human colonization.

Here, we propose a novel method to identify priority areas to conserve recent evolutionary radiations, using the Mantellidae amphibians as a case-study. We compiled a dataset of geographical observations for all mantellid taxa, and predicted their complete distribution either computing species distribution models using Maxent, for taxa with at least 10 records, or applying IUCN mapping protocols, for the remaining taxa. Using a Bayesian approach, we inferred an almost complete species-level phylogenetic hypothesis for the mantellid frogs of Madagascar, and used distribution and phylogenetic data to map species richness, phylogenetic diversity and centres of paleo- and neo-endemism. Finally, we assessed to which extent the different levels of Mantellidae diversity are already protected by the network of existing protected areas, and identified additional priority areas for conservation. In view of the proposal to extend Madagascar’s protected areas network, our results can potentially have important implications in the conservation of Malagasy amphibians.

**PROGRESS AND CHALLENGES IN RESTORING A NATIONALLY EXTINCT SPECIES: *Pelophylax lessonae* IN THE UK**

JIM FOSTER

Amphibian and Reptile Conservation Trust, Bournemouth, UK

YVETTE MARTIN

Amphibian and Reptile Conservation Trust, Bournemouth, UK

KAREN HAYSOM

Amphibian and Reptile Conservation Trust, Bournemouth, UK

The pool frog *Pelophylax lessonae* was extirpated in the UK in the 1990s. After research clarifying that the species was native to the UK, it was decided that it was both feasible and desirable to attempt reintroduction. This decision was partly informed by the finding that native UK pool frogs formed part of a distinct and vulnerable northern clade. To date there have been two UK reintroduction attempts, the first using wild-to-wild translocation of Swedish founders, and the second using head-started larvae and metamorphs sourced from the first population. Early results are encouraging, indicating the establishment of small populations with recruitment at both sites. The project has demonstrated notable successes, including: demonstration of the value of IUCN translocation guidance in project planning; translocation and head-starting as a population establishment tool; targeted habitat restoration; setting and monitoring of reintroduction targets; integration of disease risk management; partnership working. Challenges have included: resourcing and capacity to scale up to further reintroduction sites; balancing *P. lessonae* recovery objectives with those of other species and habitats; setting meaningful population size targets; refining head-starting methods; defining fine-scale habitat management objectives; reconciling landscape connectivity and disease risks. We suggest that learning points from this project may be useful for others planning or already working on amphibian reintroductions.

**AN ENDEMIC NEWT (*Calotriton arnoldi*) AS A TOOL FOR CONSERVATION OF RIVERSIDE HABITAT**

DANIEL GUINART

Montseny Natural Park, Barcelona Provincial Council, Barcelona, Spain

FÉLIX AMAT

Museu de Granollers, Granollers, Spain

SÒNIA SOLÓRZANO

Montseny Natural Park, Barcelona Provincial Council, Barcelona, Spain

JORDINA GRAU

Montseny Natural Park, Barcelona Provincial Council, Barcelona, Spain

Montseny newt (*Calotriton arnoldi*) is an amphibian endemic to the Montseny Natural Park and Biosphere Reserve. It was acknowledged as a new taxon in 2005 and, based on its small range and population size; it is considered critically endangered by the IUCN. Due to fragility of its natural population, current conservation efforts have been especially focused on improving its natural habitat and developing new research activities. Life Tritó Montseny project (LIFE15 NAT/ES/000757) is intended to promote around fifty actions to ensure *Calotriton arnoldi* conservation in five strategic lines aiming at: 1) achieving extended legal coverage and species protection as well as developing a new conservation plan; 2) building up a breeding program and reintroduction to ensure the viability of Montseny's newt populations conservation in their natural habitat; 3) reducing water withdrawn from streams, stimulate rainwater use, ecological connectivity and riparian forest restoration; 4) increasing collaboration between scientists and managers to favor knowledge transfer of *Calotriton arnoldi*'s biology and to unravel its needs and ecological threats and, finally; 5) promoting dissemination through educational and informative material, digital environment and social networks, involving local stakeholders and focusing on the conservation of the riverside habitat and its biodiversity.

## **WIND FARMS – A NEGLECTED OPPORTUNITY FOR VIPER CONSERVATION?**

JEANETTE HALL

Highland Biological Recording Group, Inverness, UK

CAMERON LAW

University of Aberdeen, School of Biological Sciences, Aberdeen, UK

LESLEY LANCASTER

University of Aberdeen, Institute of Environmental and Biological Sciences, Aberdeen, UK

SAM HANDY

Bangor University, Bangor, UK

MIKE HINCHLIFFE

University of Aberdeen, School of Biological Sciences, Aberdeen, UK

CLARE O'BRIEN

Highland Biological Recording Group, Inverness, UK

KATIE O'BRIEN

University of St Andrews School of Biology, St Andrews, UK

SAM WATTS

Bangor University, Bangor, UK

DAVID O'BRIEN

Highland Biological Recording Group, Inverness, UK

Global concern has been expressed about declines in snake populations and IUCN classes three of the seven European *Vipera* species as vulnerable. Whilst *V. berus* (adder) is classed as Least Concern, it is believed to be declining. Most British adder populations are small and fragmented, and a recently published study showed numbers to be declining. The main negative factors are human disturbance, habitat management and fragmentation. Conservation efforts usually focus on nature reserves, which are generally managed for a range of species, including birds that predate adders, and reptile reintroduction and reinforcement projects often suffer high losses to avian predators. Land-based wind farms contain large areas of habitat suitable for adders and are generally inaccessible to the public. They are managed to deter raptors, and thus may also provide refuges from predation.

Using life-sized clay models of adders, we investigated predation within a wind-farm array, and on a control site with similar topography and habitats, 1km away (beyond observed bird displacement effect distances). Attack rates at the control site were comparable with similar studies elsewhere in Europe, but were lower within wind farm arrays, although several species of predatory birds were observed within both sites. Sheep grazing severely offset this benefit. Wind farm sites may represent a neglected opportunity in reptile conservation, provided grazing is managed carefully.

**FOCUSSING ON YELLOW-BELLIED TOAD (*Bombina variegata*) POPULATIONS  
IN THE WESTERWALD REGION (GERMANY) – CONSEQUENCES FOR  
REGIONAL SPECIES CONSERVATION MANAGEMENT**

ALENA MARCELLA HANTZSCHMANN

Institute for Integrated Natural Sciences, University of Koblenz-Landau, Koblenz, Germany

ULRICH SINSCH

Institute for Integrated Natural Sciences, University of Koblenz-Landau, Koblenz, Germany

The yellow-bellied toad (*Bombina variegata*) is a threatened and strictly protected amphibian species in Germany. During the past decades, populations have declined due to loss of natural habitats as well as reproduction ponds in secondary habitats. To inform local conservation management and to develop transregional recommendations for action, we studied the demographic and genetic structure of *Bombina variegata* populations in the Westerwald region, northern Rhineland-Palatinate. This is one of the northernmost regions in which *Bombina variegata* is thought to be widely distributed, thus serving as an important reservoir for (re)colonisation. This case study aimed to estimate: (1) annual recruitment of metamorphs, (2) dispersal and (3) longevity, describing population persistence, connectivity among populations and resilience at varying reproductive success of *B. variegata* populations. From 2016 to 2018, we studied yellow-bellied toads inhabiting a former military training area near Koblenz using (1) weekly capture-mark-recapture (CMR) events during reproductive periods, (2) microsatellite analysis of genetic substructuring and (3) skeletochronology for age determination. We found a low dispersal capacity and hence fragmentation despite short distances between populations in this area. In addition, this potentially long-lived species had a very short lifespan in northern Rhineland-Palatinate. Informed conservation management of fragmented and short-lived *B. variegata* populations requires: (1) the construction of temporary and permanent ponds for reproduction, (2) stepping stone ponds for the connectivity of fragmented populations and (3) habitat management measures at regular, short intervals.



**DATA FROM LONG TERM TOAD PATROLS SHOW MODERATE DECLINE OF  
COMMON TOAD (*Bufo bufo*) IN THE NETHERLANDS**

JELGER HERDER

RAVON, Nijmegen, The Netherlands

ROLF VAN LEENINGEN

RAVON, Nijmegen, The Netherlands

Groups of volunteers organized as ‘Toad Patrols’ help amphibians to cross roads so the animals can reach their breeding ponds in spring. Since 2008 these Toad Patrols in the Netherlands are organized in the project “Padden.nu”. This project aims to facilitate the Toad Patrols to carry out their conservation work. On the website of the project participating Toad Patrols can enter their data. Since 2008 over 91 Toad Patrols with 186 transects have entered their data in this database, which provides us with an unique long term dataset with number of individuals per species per year. Using the statistical package TRIM (TRENDS and Indices for Monitoring data) we were able to calculate trends in numbers over the past ten years for the three most common species found in toad patrols: common toad (*Bufo bufo*), common frog (*Rana temporaria*) and smooth newt (*Lissotriton vulgaris*). The trend of the common toad shows a moderate decline over the past 10 years. This is in line with previous findings from the UK and Switzerland and Liechtenstein. We currently receive many ad hoc reports from the public, landowners and conservationists who report anecdotal declines of their local frog and toad populations. In our presentation we will discuss these findings and will also present the trends of common frog and smooth newt.

## **HERPETOFAUNA AND ROAD CONSTRUCTION – COMPLEX MITIGATION MEASURES**

**JAN KOVARIK**

NaturaServis s.r.o., Hradec Králové, Czech Republic

**ROMAN ROZINEK**

NaturaServis s.r.o., Hradec Králové, Czech Republic

**ANNA KOZAKOVA**

NaturaServis s.r.o., Hradec Králové, Czech Republic

**JIRI FRANCEK**

NaturaServis s.r.o., Hradec Králové, Czech Republic

The necessity of nature conservation measures in road construction is obvious and enshrined in the EU legislation. However, the proposals and implementation of these measures vary considerably from case to case, and both in scale and quality. One of our main activities are testing/developing measures to mitigate the negative impacts on the herpetofauna along line structures during construction and after commissioning. We advocate that the process is as comprehensive as possible. Based on herpetological research, drift fences with pitfall traps are installed to prevent animals from entering the hazardous construction area. Along with the barriers, the whole site, which generates appealing habitats for amphibians during the construction, is under daily supervision. However, the function of drift fences is not limited to maintaining the amphibian-free construction site, but also serves as a long-term monitoring tool. Thanks to the collected data, we install permanent barriers with underpasses on the most frequent migration routes, thus increasing the permeability of the landscape for herpetofauna. Linear structures, however, do not only fragment the habitat but also directly destroy them. Therefore, within the framework of mitigation measures, we also create new habitats. Animals are relocated here from sites that are too degraded due to construction and unsuitable for amphibian life. Here, monitoring and maintenance takes place for at least ten years. Currently we manage more than 30 km of temporary barriers on 8 sections of motorway construction, where we catch thousands of amphibians and reptiles every year.

**COST-EFFECTIVE ANALYSIS OF MITIGATION MEASURES FOR AMPHIBIAN  
PROTECTION ON ROADS**

ANNA KOZAKOVA

NaturaServis s.r.o., Hradec Králové, Czech Republic

ROMAN ROZINEK

NaturaServis s.r.o., Hradec Králové, Czech Republic

JIRI FRANCEK

NaturaServis s.r.o., Hradec Králové, Czech Republic

Various types of mitigation measures to reduce the road-mortality of amphibians are carried out annually in many places of the Europe. This work brings not only an overview of the most frequently used measures, but also a comparison of their financial demands. Based on data from year-round temporary drift fences with pitfall traps, specifically from 109 sites with 72,360 individuals, we calculated the proportion of captured individuals in each month and these results were compared to the financial costs of each type of measure. The most commonly used temporary drift fences during spring migration can capture merely 15 % of individuals at the time of spring and reverse migration only 30 % of individuals entering the hazardous area during the season. In terms of money spent, these measures are not cost-effective. The most cost-effective measure is the permanent fence with installed underpasses, which protects the moving amphibians and other small vertebrates throughout the whole season, without the need for daily control.

## **NEW LOCALITY OF *Zamenis longissimus* IN THE CZECH REPUBLIC**

ANTONÍN KRÁSA

Nature Conservation Agency of the Czech Republic, Blansko, Czech Republic

*Zamenis longissimus* is one of the rarest herp species in the Czech Republic and definitely the rarest snake species. There are three known populations living in different parts of the Czech Republic. Two of them are connected to Austrian (Podyji) and Slovakian (Karpaty) populations and are in a relatively good state since they were first discovered in 1984. The last one, Poohří, known from the 19<sup>th</sup> century, is completely isolated and thus much more vulnerable. Unfavorable status of this population was proven on the beginning of 21<sup>st</sup> century when extensive monitoring was realized. Consequently, in 2007 a National Action Plan for this species was implemented to reverse population decline and area reduction mainly in this population but also in the others. There are many positive outcomes: populations are stable and the habitat features of many localities are becoming more favourable. Moreover, a new population, situated only about 50 kilometers from the capital city of Prague, was found as a side effect of our activities;. This new finding gave us new opportunities for extensive research and activities concerning this species. Also it showed us that there are still a lot aspects to learn about biology and distributions of this big snake species even in well studied central Europe.

**BALKAN HERPS PROJECT: SPATIAL CONSERVATION PRIORITIES FOR THE AMPHIBIANS AND REPTILES IN THE BALKAN PENINSULA**

EDVÁRD MIZSEI

Hungarian Academy of Sciences, Centre for Ecological Research, Debrecen, Hungary

DANIEL JABLONSKI

Comenius University in Bratislava, Department of Zoology, Bratislava, Slovakia

DAN COGALNICEANU

University Ovidius Constanta, Faculty of Natural and Agricultural Sciences, Constanta, Romania

IOSIF RUBEN

University Ovidius Constanta, Faculty of Natural and Agricultural Sciences, Constanta, Romania

ILIAS STRACHINIS

Aristotle University of Thessaloniki, Faculty of Sciences, School of Biology, Thessaloniki, Greece

ELIAS TZORAS

Ecological Movement of Patras, Patra, Greece

TIBOR SOS

Milvus Group – Bird and Nature Protection Association, Târgu Mureş, Romania

JEROEN SPEYBROECK

Research Institute for Nature and Forest, Herman Teirlinckgebouw, Brussels, Belgium

CETIN ILGAZ

Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir, Turkey

IVONA BURIC

Association Hyla, Zagreb, Croatia

JUDIT VÖRÖS

Department of Zoology, Hungarian Natural History Museum, Budapest, Hungary

SZABOLCS LENGYEL

Hungarian Academy of Sciences, Centre for Ecological Research, Debrecen, Hungary

ZSOLT VÉGVÁRI

Hungarian Academy of Sciences, Centre for Ecological Research, Debrecen, Hungary

MIKLÓS BÁN

University of Debrecen, Department of Evolutionary Zoology, Debrecen, Hungary

MÁRTON SZABOLCS

Hungarian Academy of Sciences, Centre for Ecological Research, Debrecen, Hungary

The Balkan Peninsula is among the most biologically diverse areas in Europe. It has more than 120 species of amphibians and reptiles with several endemics. Protection of the Balkans is a high responsibility and requires sufficient and effective protected area coverage. The aim of the Balkan Herps project is to assess the representation of the regional herpetofauna under the current protected area network and reveal potential shortfalls. We created a database of distribution records ([openbiomaps.org/projects/balkanherps](http://openbiomaps.org/projects/balkanherps)) and built 30' resolution species distribution models using Bioclim and Envirem explanatory variables. We applied the systematic conservation planning tool called Zonation which ranked the sites in the study area by their importance. Generally, the Adriatic Coast, Peloponnese, Thrace and Danube Delta had higher scores while the Central Balkans had lesser. We revealed the best 17% of the area and calculated its coverage by protected areas. We found 34% coverage with obvious gaps occurring in non-EU countries, but we also found shortfalls for example in the Peloponnese and Rhodope regions. It is promising that many deficiencies can be likely solved by the designation of new Natura 2000 areas in the right places in EU candidate countries. However, there are still gaps in the Balkan Herps database, but the collaboration in progress could give a foundation for a cross-border conservation plan in the Balkan Peninsula to effectively protect herpetological diversity.

**CONSTRUCTED URBAN WETLANDS CAN SUPPORT HEALTHY AMPHIBIAN POPULATIONS BUT DO THEY BRING PEOPLE INTO CONTACT WITH NATURE?**

DAVID O'BRIEN

Highland Biological Recording Group, Inverness, UK

JEANETTE HALL

Highland Biological Recording Group, Inverness, UK

ALEXANDRE MIRÓ

Centre for Advanced Studies of Blanes, Girona, Catalonia

MARCIA RAE

Highland Council, Inverness, UK

MATTIA FALASCHI

University of Pavia, Pavia, Italy

ROBERT JEHLE

University of Salford, School of Environment and Life Sciences, Manchester, UK

Urbanisation is a major threat to many taxa including amphibians. Expanding cities take away land from biodiversity, perturb 'natural processes' such as flooding and nutrient cycling, and fragment habitats. In addition, urban citizens are less likely to experience nature and the health and well-being effects it provides. In compliance with the EU Water Framework Directive, constructed wetlands known as Sustainable Drainage Systems (SuDS) have been installed in all Scottish cities to reduce flood and pollution risk. Our study began in Inverness and expanded to a further 11 towns and cities in Scotland. We considered the value of SuDS as habitats for amphibians and other species, as facilitators of gene flow, and as places where urban people can come into contact with nature. We found that SuDS can offer new habitats for wildlife, particularly amphibians, there was no reduction in genetic diversity amongst common frog *Rana temporaria*, when compared with countryside ponds, and that there is strong gene flow between populations. Biodiversity value varied greatly between SuDS with poorer neighbourhoods tending to have wetlands of lower ecological quality, however this can be avoided through appropriate design and management. Our findings are being used in SuDS design in three Scottish cities.

## **AMPHIBIANS & ROADS - IN PURSUIT OF THE COMMON OBJECTIVES**

KATJA POBOLJŠAJ

Centre for Cartography of Fauna and Flora, Miklavž na Dravskem polju, Slovenia

In Slovenia, studies on amphibian road mortality have been carried out at the local, regional and national levels in the last 20 years. So far, there are more than 1,500 locations of roadkills registered in the country. In addition, some long-term volunteer conservation actions have been conducted for several years, with new actions starting every year. Despite all conservation efforts, only few amphibian mitigation measures have been implemented on state roads. In the light of this, identification of hotspots is a valuable tool for national planning and prioritizing locations for road mitigation measures on state roads in the future.

In 2018 we prepared the updated overview of amphibian migration across the state managed roads in Slovenia for Slovenian Infrastructure Agency (SIA). The main outcomes of the study were: a) proposal of top 13 road sections for urgent amphibian mitigation measures; b) setting up the national methodology for decision making for setting up mitigation measures; c) proposal of monitoring methods for effectiveness of mitigations. The follow up is preparation of technical guidelines for the planning, design and evaluation of amphibian mitigation measures for Slovenian state roads, adopted by SIA. Background document for amphibians is in preparation in 2019. I review the process of latest development of amphibian mitigation measures in Slovenia, from both amphibian conservation and road management point of view. Finally, I discuss conceptual frameworks for mitigation, involving of volunteer actions (toad patrols) and potential development of road mitigation projects in relation to the existing recent developments on national level.

## DECLINE IN AMPHIBIAN ABUNDANCE AND BIOMASS IN NIEPOŁOMICIE FOREST (S POLAND) OVER A 50 YEAR PERIOD

MACIEJ PABIJAN

Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland

SARA BAŃK

Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland

MACIEJ BONK

Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland

WIOLETA OLEŚ

Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland

WERONIKA ANTOŁ

Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland

BARTŁOMIEJ ZAJĄC

Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland

STANISŁAW BURY

Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland

IZABELA SADZA

Towarzystwo Herpetologiczne Matrix, Wrocław, Poland

There is a dearth of information on amphibian abundance in terrestrial environments. Our aim was to describe changes in amphibian abundance and biomass in a deciduous forest in southern Poland (Niepołomice Forest) by comparing data collected in 2016-2017 with historical data from 1967-1968. Abundance data were obtained by removal sampling of 30 m x 30 m terrestrial plots. Five plots were sampled in 1967 – 1968; 16 plots were sampled in 2016 – 2017. We used a Bayesian method that takes into account variation in catchability among individuals to estimate abundance. We found that amphibian abundance decreased at least 4-fold over the study area (from  $253.6 \pm 69.9$  to  $60.7 \pm 45.8$  individuals per plot), with a 20-fold decline detected for plots situated directly in the historic sampling area. A similar decline was noted for amphibian biomass, declining from 12.4 kg/ha (range 11.8-13.2 kg/ha) to 4.3 kg/ha (range 0.2-7.2 kg/ha). We also noted changes in anuran community structure: in 1967-1968 *Bufo bufo* dominated (65% of total catch), followed by *Rana arvalis* (18%), *R. temporaria* (11%), *Pelophylax lessonae* (3%) and *Bombina bombina* (2%). In 2016 – 2017, *R. temporaria* was most common (82%), followed by *B. bufo* (13%) and *R. arvalis* (4%). Our data reveal a significant decline in an anuran community in a relatively intact fragment of Central European lowland deciduous forest. We hypothesize that a drop in ground water levels and road mortality are the main culprits behind the decline. Funded by a SEH Grant in Herpetology - Conservation.



## **LOWERING *Bd* PREVALENCE AS A PART OF MITIGATION MEASURES**

ROMAN ROZINEK

NaturaServis s.r.o., Hradec Králové, Czech Republic

JAN KOVARIK

NaturaServis s.r.o., Hradec Králové, Czech Republic

ANNA KOZAKOVA

NaturaServis s.r.o., Hradec Králové, Czech Republic

JIRI FRANCEK

NaturaServis s.r.o., Hradec Králové, Czech Republic

Due to planned development of nuclear power plant Temelín, the company NaturaServis has been providing mitigation measures for amphibian and reptiles population. At the planned development site, a wetland was present, occupied by 12 species of amphibians and 4 species of reptiles on an 8-hectare area. During 2014, NaturaServis created 78 new ponds in a greenfield site, about 5 km away from the original habitat. For reptiles, artificial hibernation and aestivation shelters, stonewalls and egg-laying areas were created. In the meantime, we investigated *Bd* presence in the area of interest. In 2015 the company initiated transfers of animals to new habitats, continued till the end of 2017 season for a total number of around 115 000 individuals. Species susceptible to *Bd* were tested before releasing. Positive individuals were kept in artificial tanks designed by our company until they were cured. The bottom of these tanks is sloping and covered by black foil. It allowed us to create aquatic habitat, or both aquatic and terrestrial habitats. The success rate of this therapy was almost 100%. Individuals who underwent this cure and then tested negatively for *Bd* were then released to new ponds. We designed management of these new habitats on the basis of experience with therapy in artificial basins. That means we preserve many insolated shallows, which should hopefully help to keep the *Bd* prevalence in new habitats as low as possible. Monitoring showed only 2 pools in which the disease is present and all the species transferred have successfully reproduced.

**HERPETOFAUNA CONSERVATION IN THE LIGHT OF INTERNATIONAL  
TRADE: THE PERSPECTIVE OF GOVERNMENTAL CONSERVATIONISTS**

MONA VAN SCHINGEN

Federal Agency for Nature Conservation (CITES Scientific Authority), Bonn, Germany

HAI NGOC NGO

Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, Hanoi Vietnam

TRUONG QUANG NGUYEN

Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (CITES  
Scientific Authority), Hanoi Vietnam

THOMAS ZIEGLER

Cologne Zoo, Cologne, Germany

The European Union is a major importer of amphibians and reptiles and thus is responsible for the conservation also of species outside its range. Amphibians and reptiles are globally traded in large volumes to supply the international demand for pets, skin, food and traditional medicine. Unsustainable commercial exploitation has contributed to population declines and local extirpations of numerous species worldwide. Endemic and range restricted species are especially vulnerable. The Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES) provides the key tool to regulate and allow sustainable international trade in listed species. Currently, about 8% of described reptile species and about 2% amphibian species are listed in the Appendices of the Convention. However, the implementation of CITES poses major challenges to relevant stakeholders in importing and exporting countries. Using endangered Asian lizards as example, this talk presents current activities and major challenges of the German CITES authorities. Detailed knowledge on the ecology, distribution and population status is essential to evaluate the need to include species in the CITES Appendices as well as to allow sustainable trade in and conservation of wild populations. In this context a strong cooperation between scientists and Governmental authorities is of particular importance.

## HOW (AND WHY) TO DETECT INFECTIOUS DISEASES?

ANNEMARIEKE SPITZEN – VAN DER SLUIJS

Reptile, Amphibian and Fish Conservation the Netherlands, Nijmegen, the Netherlands

TARIQ STARK

Reptile, Amphibian and Fish Conservation the Netherlands, Nijmegen, the Netherlands

In all invasion stages of emerging infectious (amphibian) diseases, passive surveillance and syndromic surveillance organised via an Early Warning System (EWS) is a powerful, economic and useful tool to detect an outbreak and follow-up on the impact and severity. In the Netherlands we currently maintain an extensive network of people who are on alert to report dead, sick or aberrant amphibians. This has led to the discovery of ranavirosis in the north of the Netherlands and a mass mortality event of the rare midwife toad (*Pelobates fuscus*) due to a ranavirus infection, as well as the observation of the decline of the fire salamander (*Salamandra salamandra*) due to *Batrachochytrium salamandrivorans* (Bsal). Our work shows, albeit preliminary, that an EWS provides insight into the distribution of various pathogens, such as herpes, Amphibiotecum, Saprolegnia and *B. dendrobatidis*, and may therefore be considered the main initial source of information for detecting an impact of pathogens on wild amphibian populations. We therefore suggest more resources should be allocated to the set-up of Early Warning Systems to obtain data from remote locations.

**CONSERVATION GENETICS OF *Bufo viridis* (ANURA: BUFONIDAE) IN THE UPPER RHINE VALLEY.**

JEAN-PIERRE VACHER  
Association BUFO, Strasbourg, France  
CINDY GERARD  
Association BUFO, Strasbourg, France  
SYLVAIN URSENBACHER  
NLU, Basel, Switzerland

The green toad *Bufo viridis* reaches its western limit of distribution in northeastern France, where it is listed as an endangered species. There, this species occupies secondary habitats like quarries, agricultural and industrial lands, and road ponds. The localities occupied are highly fragmented by a dense network of roads, and also by ever-growing urban areas. A first population genetic approach was carried out in 2011, using microsatellite markers. This study showed that the population located west of Strasbourg is in fact constituted by two well-differentiated genetic clusters. These clusters were disconnected with another southernmost cluster found in the vicinity of Mulhouse. Since then, new localities were found more to the north and to the south, suggesting good population dynamics of the species despite apparent fragmentation. We wanted to test whether the newly discovered populations were linked to only one cluster or to several ones, and if they were still disconnected. We also wanted to test the barrier effect of the Rhine River on green toad populations. We genotyped 563 toads (324 in 2011 and 239 in 2018) from Alsace and Baden-Württemberg with seven microsatellite markers to characterize their genetic structure. Our results show that populations in France and Germany separated by the Rhine are strongly differentiated. We also found that all new populations found around Strasbourg are linked to only one cluster. Finally, all the populations around Mulhouse are disconnected, suggesting strongly fragmented populations in this area. Consequently, we recommend conservation priorities for the upcoming years focalizing on habitat reconnections.

**OPTIMIZING THE CREATION OF NEW WILD POPULATIONS OF THE  
MONTSENY BROOK NEWT (*Calotriton arnoldi*)**

DANI VILLERO

Forest Science and Technology Centre of Catalonia, Solsona, Spain

FÉLIX AMAT

Montseny Natural Park, Barcelona Provincial Council, Fogars de Montclús, Spain

STEFANO CANESSA

Wildlife Health Ghent, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

DANIEL GUINART

Montseny Natural Park, Barcelona Provincial Council, Fogars de Montclús, Spain

VIRGILIO HERMOSO

Forest Science and Technology Centre of Catalonia, Solsona, Spain

JOSÉ SALGADO ROJAS

Forest Science and Technology Centre of Catalonia, Solsona, Spain

SONIA SOLORZANO

Montseny Natural Park, Barcelona Provincial Council, Fogars de Montclús, Spain

The Montseny brook newt (*Calotriton arnoldi*) is a Critically Endangered amphibian with fewer than 1500 adults spread over seven populations in different streams within the Montseny massif, in Catalonia (NE Spain). Although wild populations of the Montseny brook newt are under the umbrella of different protection instruments (Natura 2000, Natural Park and Biosphere Reserve), the streams where it occurs are threatened: often located on private land, with considerable forestry activity or bottled water (27% of all bottled water in Spain come from this area). Because of such fragility of natural populations, conservation efforts to date have focused on consolidating a captive breeding programme. To ensure the long-term survival of wild populations and expand the distribution range of the species, complementary measures have also been considered, such as the creation of new wild populations with the specimens coming from captive breeding. For this purpose, and to maximize the success of newly established populations, we have conducted an optimization analysis taking into account population dynamics at potential new sites and the risks to the viability of these populations (e.g. forestry, water catchments, food availability, presence of predators, etc.), also including the risk of infection and spread of the fungus *Batrachochytrium salamandrivorans*, recently detected in the region. The optimization algorithm we developed allows managers to identify the most suitable sites to create and reinforce new populations over the next 10-15 years, based on the population stocks planned for the coming years from the captive breeding programme.





# Session: Ecology and Ethology

## **EFFECT OF TEMPERATURE ON THE VISUAL DISPLAYS OF THE JACKY DRAGON**

MARCO D. BARQUERO

Sede del Caribe, Universidad de Costa Rica, Limón Costa Rica

RICHARD PETERS

Department of Ecology, Environment & Evolution, La Trobe University, Melbourne, Australia

MARTIN J. WHITING

Department of Biological Sciences, Macquarie University, Sydney, Australia

In ectotherms such as lizards, temperature is a key determinant of their behavior and their ability to signal to conspecifics. We asked whether changes in the thermal environment along the distribution of an agamid lizard, the Jacky dragon (*Amphibolurus muricatus*), modifies visual signal properties (frequency and duration). We first collected temperature data from 12 weather stations to understand variation in temperature across the species' range. We then collected field body temperatures ( $T_b$ ) for three populations before measuring preferred body temperatures ( $T_{pref}$ ) in the lab. Finally, we examined the role of temperature on the frequency and duration of display behavior at low (28 °C) and high (35 °C) temperatures consistent with what individuals experience in the wild. We found differences of 5.6-9.4 °C in mean air temperature across sites of the species' range. Field body temperatures, but not preferred body temperatures (overall average = 32.4 °C), differed among populations, with individuals from one population attaining higher temperatures than the other two. We also found a positive relationship between air temperature and the number of displays used during intraspecific communication, as well as differences in the number of displays during high- and low-temperature treatments (more displays during the high-temperature treatment). We suggest that social signaling is plastic and able to respond to local conditions such that variation of body temperatures mirrors that of the thermal environment and in turn, influences the frequency and duration of Jacky dragon visual signals. Display behavior is therefore dependent on environmental temperature and future changes to the thermal environment could have an impact on communication through its effect on display duration and frequency.



**HOW MANY SHADES OF YELLOW? FACTORS DETERMINING THE DORSAL COLORATION PATTERN OF THE FIRE SALAMANDER (*Salamandra salamandra*)**

BENEDETTA BARZAGHI

Department of environmental sciences, University of Milan, Milan, Italy

ANDREA MELOTTO

Department of environmental sciences, University of Milan, Milan, Italy

PAOLA COGLIATI

Department of environmental sciences, University of Milan, Milan, Italy

GENTILE FRANCESCO FICETOLA

Department of environmental sciences, University of Milan, Milan, Italy

RAOUL MANENTI

Department of environmental sciences, University of Milan, Milan, Italy

One of the most interesting features of European salamandrids of the *Salamandra* genus is represented by their dorsal pattern. The coloration is due to the different disposition of the chromatophores that characterize the epidermic cells. In fire salamanders the typical yellow and black aposematic pattern is particularly evident. The aim of this study is to evaluate the variability of *S. salamandra*'s dorsal pattern between populations belonging to different altitudes and habitats with different availability of trophic resources. In particular we studied both adults and metamorphosed from 27 populations living in different piedmont, hilly and mountains areas of the Italian Prealps. For each population we both collected dorsal pictures of adults during multiple night surveys and we collected 10 larvae from the main breeding site. Half larvae were reared at "ad libitum" conditions and half were reared at poor nutritional conditions; after 45 days from metamorphosis we collected pictures of their dorsal pattern. For each population we also estimated the maximum density of available earthworms. From the pictures we extracted all the yellow pixels and we measured HSV mean values of each individual. For both juveniles and adults, the results showed a strong correlation between dorsal pattern coloration and trophic resources. On the contrary we did not detect any effect of the population origin in determining HSV yellow features. Our results suggest that yellow pattern coloration of fire salamander is a relatively plastic and costly feature, strongly affected by the trophic resources available during both adult and larval stages.

**TURNING, TUGGING, RUNNING AND REVERSING: SURVIVAL STRATEGIES OF  
THE EUROPEAN POND TURTLE**

VUKAŠIN BJELICA

Faculty of Biology, University of Belgrade, Belgrade, Serbia

MARKO MARIČIĆ

Faculty of Biology, University of Belgrade, Belgrade, Serbia

ANA GOLUBOVIĆ

Faculty of Biology, University of Belgrade, Belgrade, Serbia

The European pond turtle, although mostly aquatic, still has to face a potential terrestrial predator while migrating or laying eggs. Getting stuck in vegetation is also possible, both on land and underwater, leaving the animal vulnerable to predators and exposure. We measured how animals from three populations behaved during timed test that imitate real – life situations they could face when evading a predator or freeing themselves from dense vegetation. All turtles had faster reaction times when placed on their plastron, suggesting that the upright position is safer and offers easier escape then when turtles are overturned on their carapace. On the other hand, sexes were equally fast to self-right from an overturned position, as well as to release from ‘‘vegetation’’ stuck on their plastron. We found significant negative correlation between body size and efficiency of self-righting and realizing from ‘‘vegetation’’. What this could imply is that smaller body size probably leads to greater agility and mating success, which is in accordance with the general hypothesis on sexual size dimorphism suggested for several other turtle species. Although females are generally the larger sex in European pond turtles, we found no effect of body size on their agility performances. Interestingly, differences in performances between sexes were much more expressed than among the three tested populations.

**DENSITY-DEPENDENT HABITAT SELECTION PREDICTS FITNESS AND  
ABUNDANCE IN A SMALL LIZARD**

GABRIEL BLOUIN-DEMERS

University of Ottawa, Ottawa, Canada

JAMES E PATERSON

University of Ottawa, Ottawa, Canada

Density-dependent habitat selection has been used to explain patterns of abundance of species between habitats. Thermal quality, a density-independent component of habitat suitability, is often the most important factor for habitat selection in ectotherms. Ectotherms may reach high densities such that individual fitness is reduced in a habitat due to increased competition for finite resources. Using ornate tree lizards (*Urosaurus ornatus*) at ten sites each straddling two adjacent habitats (wash and upland), we tested the hypothesis that habitat selection is density-dependent even when thermal quality differs between habitats. We first tested that fitness proxies decline with density in each habitat, indicating density-dependent effects on habitat suitability. We also confirmed that the two habitats vary in suitability (quantified by food abundance and thermal quality). Next, we tested the predictions that habitat selection depends on density and that fitness proxies are equal in the two habitats within a site. We found that monthly survival rates decreased with density, and that the wash habitat had more prey and higher thermal quality than the upland habitat. Lizards preferred the habitat with more food and higher thermal quality, lizard densities in the two habitats were positively correlated, and fitness proxies of lizards did not differ between habitats. These patterns are consistent with density-dependent habitat selection, despite differences in thermal quality between habitats. We expect that density-dependent habitat selection is widespread in terrestrial ectotherms when densities are high and temperatures are close to their optimal performance range.

**INFECTION OF PARTHENOGENETIC LIZARDS BY BLOOD PARASITES DOES NOT SUPPORT THE “RED QUEEN HYPOTHESIS” BUT REVEALS THE COSTS OF SEX**

MARINE ARAKELYAN

Faculty of Biology, Yerevan State University, Alek Manoogian 1, 0025, Yerevan, Armenia

TEHMINE HARUTYUNYAN

Faculty of Biology, Yerevan State University, Alek Manoogian 1, 0025, Yerevan, Armenia

SARGIS A. AGHAYAN

Scientific Center of Zoology and Hydroecology, Sevak str 7, 0014, Yerevan, Armenia

MIGUEL A. CARRETERO

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

Sexual organisms should be better suited than asexual ones in a context of continuous evolution in response to opposite organisms in changing environments (“Red Queen” hypothesis of sex). However, sex also carries costs associated with the maintenance of males and mating (sex cost hypothesis). Here, both non-mutually excluding hypotheses are tested by analysing the infestation by haemogregarines of mixed communities of *Darevskia* rock lizards composed of parthenogens generated by hybridisation and their sexual relatives. Prevalence and intensity were recorded from 339 adult lizards belonging to six species from five syntopic localities and analysed using Generalized Mixed-Models (GLMM). Both infestation parameters depended on host-size (like due to longer exposure with age), sex and, for intensity, species. Once accounting for locality and species, males were more parasitized than conspecific females in sexual species, but no signal of reproductive mode itself on parasitization was recovered. Essentially, male-male interactions increased haemogregarine intensity while females either sexual or asexual had similar reproductive costs when in the same conditions. These findings deviate from the predictions from “Red Queen” dynamics while asymmetric gender costs are here confirmed. Thus, increased parasitization pressure on males adds to other costs, such as higher social interactions and lower fecundity, to explain why parthenogenetic lizards apparently prevail in the short-term evolutionary scale. How this is translated in the long-term requires further phylogenetic analysis.

**CLIMATIC PREFERENCES OF A SAND LIZARD POPULATION (*Lacerta agilis*  
LINNAEUS 1758) IN WESTERN GERMANY**

VIC CLEMENT

Zoological Research Museum Alexander Koenig, Bonn, Germany

RIEKE SCHLUCKEBIER

Zoological Research Museum Alexander Koenig, Bonn, Germany

JULIA PLATZEN

Zoological Research Museum Alexander Koenig, Bonn, Germany

DENNIS RÖDDER

Zoological Research Museum Alexander Koenig, Bonn, Germany

Understanding the climatic factors influencing a species behavior is important for a multitude of reasons including conservation planning. Climate ecology of *Lacerta agilis* has been studied thoroughly at the edges of its distribution range and at high elevations but hardly in the central parts. As preferences between populations living in extreme conditions can vary considerably from those of populations in more moderate conditions, in order to properly conserve central populations of *Lacerta agilis*, we cannot rely on studies conducted in extreme conditions. We therefore use a visual encounter study on a sand lizard population in a heathland near Cologne over two years and combine it with the corresponding data of a nearby weather station. Our aim is to determine climatic preferences within the population and identify which factors influence activity in sand lizards using among other methods CART models and analysis of variance. Furthermore, we aim to deliver a method which can easily be included in any visual encounter based fieldwork.

**MORPH-SPECIFIC PATTERN OF AGGRESSION THROUGHOUT THE SEASON  
IN *Podarcis muralis***

ALAN J. COLADONATO

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Italy

MARCO MANGIACOTTI

Museo Civico di Storia Naturale, Milano, Italy

STEFANO SCALI

Museo Civico di Storia Naturale, Milano, Italy

MARCO A. L. ZUFFI

Museo Civico di Storia Naturale e del Territorio, Calci (Pi), Italy

CARLOTTA PASQUARIELLO

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Italy

CRISTIAN MATELLINI

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Italy

SIMONE BURATTI

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Italy

MARA BATTAIOLA

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Italy

ROBERTO SACCHI

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Italy

The persistence of colour polymorphism (CP) within a given population is generally associated with the coexistence of alternative reproductive strategies, each one involving specific trade-offs among behavioural, morphological, physiological, and other life history. Trade-offs involving immune system are of particular interest, since the ability of dealing with parasites and diseases has severe consequences on the fitness individuals may achieve, but also entails substantial costs for them. T-level fosters a strong motivation to defend territories against rivals (i.e. increasing movements, activity, and aggressive display) and increases mating. At the same time, high T-level can decrease immune functions, favours parasite infections, stimulates risky behaviours, and thus diminishes survival, according Immunocompetence Handicap Hypothesis (ICHH), and there is no way to maximize stamina and aggressive behaviours at once. Common wall lizard (*Podarcis muralis*) showing CP in three main color (yellow, white and red) and a morph specific pattern for both immunocompetence and seasonal variation of T-levels, where the former show low stamina with high plasma T-levels and the following high stamina with low intra-sexual competitive ability. We hypothesised the presence of 2 strategies: one from yellow-morph and another from white/red-morph. Thus we tested the aggressive response to conspecifics of yellow and white-morph using a mirror inserted into the own setting box, throughout the breeding season (128 experiments). All lizard was tested after a period of acclimatization. Results demonstrated the yellow males was more aggressive in the early bird season and with a decrease towards the end, whereas white males show an opposite pattern.

**DOES SYMPATRY INFLUENCE THE PARASITE PREVALENCE AND PARASITE  
LOAD IN TWO COMPETING LACERTIDS?**

URBAN DAJČMAN

University Ljubljana, BIOTECHNICAL FACULTY, department of biology, Ljubljana, SLOVENIA

ANA PERERA

CIBIO/InBIO Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, PORTUGAL

MIGUEL A. CARRETERO

CIBIO/InBIO Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, PORTUGAL

ROK KOSTANJŠEK

University Ljubljana, BIOTECHNICAL FACULTY, department of biology, Ljubljana, SLOVENIA

ANAMARIJA ŽAGAR

National Institute of Biology, Ljubljana, SLOVENIA

Parasites are important drivers shaping animal populations by affecting growth, performance, reproductive success and survival. In sympatric related host species, parasites may shape their interspecific interaction and vice-versa - the intensity of parasite infection and parasitemia could be influenced by beforementioned interactions. Members of the genus *Hepatozoon* (Apicomplexa, Adeleorina) are the most common and widely distributed intracellular parasites of lizards. In this work we studied the parasitization patterns of two lizard species, *Iberolacerta horvathi* and *Podarcis muralis*, ranging Slovenia either in sympatry or in allopatry. We used the 18S rRNA gene to confirm parasite identity and identify potential new haplotypes and estimated prevalence and intensity using microscopy. Then, we tested for the effect of host species, geographic region, body size sex and sympatry/allopatry on these parameters. Preliminary results show significant differences in parasite prevalence and intensity among localities, as well as differences in parasite intensity both among localities and host species. This is one of the first studies examining the blood parasites in Slovene reptiles combining microscopy and molecular methods, and as such expands our knowledge on parasite diversity, their possible impact in this region and the interaction patterns between both lizard species. In addition, the study recovers the variation between sympatry and allopatry as a way of understanding coexistence of two lizard species occupying a similar ecological niche.

**CONSERVATION ECOLOGY OF CRESTED NEWTS:  
HIGH SITE INFIDELITY IN A NETWORK OF SMALL PONDS**

MATHIEU DENOËL

University of Liège, Liège, Belgium  
FNRS-FNRS Research Director

HUGO CAYUELA

Laval University, Québec, Canada

The crested newt, *Triturus cristatus*, is an emblematic and endangered amphibian species that is usually associated with large ponds, considered site faithful within a breeding season, and functioning in metapopulations. However, such large newt species can also be found in apparently suboptimal patches such as small-sized ponds (pools). Therefore, we aimed at better understanding its habitat use and dispersal strategies by innovative capture-mark-recapture using RFID aquatic telemetry in more than 100 shallow ponds from a protected area in Belgium. Data were analysed with multi-event models. We found out that such networks of small ponds can host thousands of crested newts. The population was composed of a highly pond faithful phenotype and a dispersing phenotype. Breeding site infidelity occurred at a high rate in both sexes within and between reproductive seasons. Most movements occurred between close ponds with few recaptures at the periphery of the study area. Our work highlights that in the presence of alternative aquatic habitats, newts exhibit not only habitat complementation (i.e. land and water) but also habitat supplementation (pond shift) and this, during the peak of the reproduction. These results have implications for our understanding of reproductive strategies of newts but also for their conservation. First, this shows that (apparently) suboptimal breeding habitats can be worth for conservation of crested newts when they are closely connected together and, second, very short distances among ponds (i.e. much under the « 400 m » usual target) can be important to sustain large newt populations.



## **YOU CANT'T ALWAYS GET WHAT YOU WANT – FEMALE MATE CHOICE IN THE EUROPEAN COMMON FROG**

CAROLIN DITTRICH

Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany

MARK-OLIVER RÖDEL

Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany

Mate choice in amphibians is often considered to be male based. It is assumed that in explosive breeding anuran species scramble competition precludes female choice and males are the choosy sex. In general all males should prefer to mate with a large female, because these usually produce more eggs and therefore the fitness of the successful male would increase. In a former publication we have shown that smaller *Rana temporaria* males are faster grabbing a female which could be a sign of indiscriminate mate choice behavior. This could be due to large males having advantages during scramble competition. In the current study we studied pair formation in the European common frog without competition. We conducted mate choice experiments placing a male and two differently sized female in a box and recorded their mating behavior. We found that mate choice by large males was random. There was no preference for larger over smaller females (or vice versa). In contrast small males on the other hand preferred larger females. More important, females apparently could choose their mates alike. We recorded two different release calls emitted by females when grasped by an unwanted male. Females could terminate amplexus by applying different strategies. They either turned their bodies to free themselves or they literally played dead until released. So far, death feigning as a strategy to avoid or finish mating has only been described in invertebrates and one newt species.

**METABARCODING AS A TOOL TO DETERMINE FEEDING BEHAVIOUR – IS  
THE EUROPEAN POND TURTLE A THREAT FOR OTHER ENDANGERED  
SPECIES ?**

CHARLOTTE DUCOTTERD

La Maison de la Rivière, Tolothenaz, Switzerland  
University of Lausanne, Lausanne, Switzerland  
Centre Emys, Chavornay, Switzerland

JULIEN CROVADORE

Laboratoire Plantes et Pathogènes, Lullier, Switzerland

FRANÇOIS LEFORT

Laboratoire Plantes et Pathogènes, Lullier, Switzerland

JEAN-FRANÇOIS RUBIN

La Maison de la Rivière, Tolothenaz, Switzerland

SYLVAIN URSENBACHER

Infofauna-CSCF&karch, Neuchâtel, Switzerland

Knowledge of feeding strategy and food preferences is one of the milestones of the natural history of a species and is essential to optimise conservation programs. The European pond turtle (*Emys orbicularis*, L. 1758) is the only freshwater turtle living in Switzerland and ranked as critically endangered on the Swiss Red List. Its diet is still unclear, considered by some studies as carnivorous, often scavenger and sometimes vegetarian. We conducted analyses of *Emys*' diet by developing a new method of global DNA amplification and metabarcoding analysis, using universal PCR primers to determine the species occurring in the faeces. The analysis of the diet of this species was conducted during its whole activity period (April to September) in the natural reserve of Moulin de Vert (Geneva, Switzerland) to determine if there is a shift in food intake during whole activity period and if diet varies between adults/juveniles and males/females. Furthermore, four different populations were sampled during the month of July to detect possible difference in food consumption. Moreover, this study not only determined the nutritional needs for the European pond turtle, but also demonstrated that this species is not a threat to its environment (predation on other threatened species such as amphibians). Globally, we were able to demonstrate, using the European pond turtle, that the genetic analyses of faeces could be an efficient tool to determine trophic networks with a very high level of precision.

## **INVASIVE SPECIES OVERRIDE HABITAT CHANGE IN DETERMINING NEWT DECLINE AT THE REGIONAL SCALE**

MATTIA FALASCHI

Department of Environmental Science and Policy, Università degli Studi di Milano, Milan, Italy

MARTINA MURARO

Department of Environmental Science and Policy, Università degli Studi di Milano, Milan, Italy

FEDERICO FARACI

Department of Environmental Science and Policy, Università degli Studi di Milano, Milan, Italy

FRANCESCO BELLUARDO

CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Vairão, Portugal

Department of Biosciences, Università degli Studi di Milano, Milan, Italy

MATTEO DI NICOLA

Department of Environmental Science and Policy, Università degli Studi di Milano, Milan, Italy

RAOUL MANENTI

Department of Environmental Science and Policy, Università degli Studi di Milano, Milan, Italy

GENTILE FRANCESCO FICETOLA

Department of Environmental Science and Policy, Università degli Studi di Milano, Milan, Italy

Univ. Grenoble Alpes, CNRS, Univ. Savoie Mont Blanc, Laboratoire d'Écologie Alpine (LECA), Grenoble, France

The current biodiversity crisis is caused by the joint effects of multiple processes such as habitat modifications, climate change, spread of diseases, and introduction of invasive species. The relative importance of these drivers can vary through time, but the long term studies needed to identify changes of threats through time remain scarce. In this work, we analysed 22 years of site occupancy data from 63 wetlands suffering the introduction of invasive crayfish, in order to investigate the role of microhabitat, landscape change, and invasive species, in determining the decline of two newt species through time. We performed repeated monitoring to assess the changes in occupancy by newts (*Triturus carnifex* and *Lissotriton vulgaris*) in Northern Italy. Initial occupancy of newts was negatively affected by landscape alteration (i.e. urban and agricultural cover), and by the presence of fish. Both species suffered a strong decline in the study period, with a net loss of site occupancy of 13%–19% along the study period. In 2009 the red swamp crayfish *Procambarus clarkii* was introduced in the study area. After the crayfish invasion, the main drivers of populations dynamics sharply shifted, and occupancy changes were not related to landscape or microhabitat features, as the strongest predictor of local extinctions was the colonisation of wetlands by invasive crayfish. If we want to properly identify conservation priorities under prolonged environmental changes, we need long-term data on the occurrence of both species and threats and we also have to consider how the main threatening factors can change over time.

**FIRST OBSERVATION OF CONVERGENT CHARACTER DISPLACEMENT IN A SYNTOPIC COMMUNITY OF TWO ITALIAN LIZARDS: *Podarcis muralis* AND *P.siculus***

ANDREA GINI

Scuola Normale Superiore, Pisa, Italy

MARCO A. L. ZUFFI

Museo di Storia Naturale dell'Università di Pisa, Calci, Italy

CHIARA VITILLO

Museo di Storia Naturale dell'Università di Pisa, Calci, Italy

ANDREA MASSAGLI

Museo di Storia Naturale dell'Università di Pisa, Calci, Italy

STEFANO SCALI

Museo Civico di Storia Naturale, Milano, Italy

ROBERTO SACCHI

Dipartimento di Scienze della Terra e dell'Ambiente, Pavia, Italy

ALAN J. COLADONATO

Dipartimento di Scienze della Terra e dell'Ambiente, Pavia, Italy

The co-occurrence of the lizards *Podarcis muralis* and *P. siculus* is frequent but anecdotally reported in the literature, mostly as side notes of their complex ecology and life history. There are a few studies on the interactions between these two species, but these are old or focalized only on individual populations. This research aims to fill and enlighten the possible competitive interactions between *P. muralis* and *P. siculus* when they share a microhabitat. Being a base research, which is not founded on previous works, every result will be an important contribution to the study of these two lizards. We have observed a clear trend in *P. muralis* associated with the elongation of the jaw in the syntopic population of Calci, while their body size remains unchanged between syntopic and allopatric populations. This differentiation causes an overlap in jaw length between the two species and the sharing of the same range of variation for this character. Hence, in syntopy, they are not distinguishable at all if we base our discrimination only on the length of the jaw, contrary to the common situation where *P. siculus* head-length outmeasures *P. muralis*. Syntopic *P. muralis* are instead well discriminable from their allopatric populations. For the first time, we provide comparative data from different populations, both in allopatry and in syntopy. Our preliminary results suggest a diversification only in head morphology (convergent character displacement) in the area of co-occurrence, hence a proxy of a history of co-evolution that shape the relationship of these two species.

## INFLUENCE OF COPPER ON FROGS' EARLY DEVELOPMENT

OLGA JOVANOVIĆ GLAVAŠ

Department of biology, University of Osijek, Osijek, Croatia

ŽELJKA LONČARIĆ

Department of biology, University of Osijek, Osijek, Croatia

NIKOLINA STJEPANOVIĆ

Department of biology, University of Osijek, Osijek, Croatia

BRANIMIR HACKENBERGER KUTUZOVIĆ

Department of biology, University of Osijek, Osijek, Croatia

Amphibians are the most threatened group of vertebrates and as such are of great interest in ecotoxicological research. In the latest years, utilization of nanomaterials is more widespread and due to that, the chance for its presence in the environment is increasing daily. One of these nanoparticles is copper which is used, among others, in certain pesticides and as such can easily find its way into the environment. In our study we examined the influence of nano and bulk copper on frog embryos and larvae. We tested the influence of a single exposure, at the beginning of the experiment, of four different concentrations of nano (declared size 40-60 nm) and bulk (ionic) copper on egg clutches of agile frog, *Rana dalmatina*. Each concentration was examined in triplicate, with 30 eggs each. Eight days after the last tadpole hatched, we homogenized tadpoles from each replicate and analyzed protein, lipid and glucose content and compared it to the control treatment. Our results show clear trend in increase of protein content with the increase of concentration in both nano and bulk treatment. The opposite trend is evident in carbohydrate content with the control treatment having higher recorded values, than both nano and bulk treatments. Content of lipids varied within nano treatment and was comparable to the control, and bulk treatment had lower lipid content than both, control and nano treatment. All these results suggest possible negative influence of nano and bulk copper on tadpole growth and metabolism.

**DIFFERENCES IN BEHAVIOUR OF TWO LACERTIDS IN COMPETITIVE  
RELATIONSHIP: *P.siculus* AND *P.melisellensis***

MARKO GLOGOŠKI

Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia  
SOFIA BLAŽEVIĆ

Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia  
KSENIJA HOCENSKI

Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia  
DUJE LISIČIĆ

Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia

Competition between species can lead to ecological and evolutionary differentiation of species. The Italian wall lizard (*Podarcis siculus*) is a generalist species with broad ecological tolerance and frequently excludes other small lizards. The areals of *Podarcis siculus* and *Podarcis melisellensis* sometimes overlap and when that happens *P. siculus* overpowers *P. melisellensis*. In order to better understand the behaviour of both species and to explore whether differentiation in behaviour can explain dominant-subordinate interaction between these two species, we tested 28 individuals of each species, of both sexes, in open field and radial maze. Both experiments lasted 15 to 23 minutes and were repeated 3 times in order to habituate the lizards to the new environment. Lizards' performance in novel and familiar habitat (after habituation) was measured and analysed for potential dispersal and explorative behaviour, cautiousness, risk to predation and food consumption. We hypothesized that *P. siculus*, as a dominant competitor, would show greater dispersal and explorative behaviour, be more cautious and less prone to predation and would consume more food. As expected, *P. siculus* showed greater dispersal and explorative behaviour in both open field and radial maze and ate more food. Also, *P. siculus* was more cautious and less prone to predation. These results show that different behaviour can help competitors persevere and outcompete other individuals in ecologically competitive relationships.

**TWENTY-FOUR YEARS, FOURTEEN THOUSAND TOADS:  
AN INTERIM REPORT OF A POPULATION STUDY ON  
*Bombina variegata***

GÜNTER GOLLMANN

University of Vienna, Department of Theoretical Biology, Vienna, Austria

BIRGIT GOLLMANN

University of Vienna, Department of Limnology and Bio-Oceanography, Vienna, Austria

Since 1996 we investigate population ecology of yellow-bellied toads, *Bombina variegata*, in Lainzer Tiergarten, a large nature reserve in the Vienna Woods. We use individual variation of belly patterns for registration of recaptures, and record distribution of eggs and larvae in space and time. In this near-natural habitat *B. variegata* is a prolonged breeder, spawning from April to July or August in a variety of water bodies, including streams. So far, we have registered over 70.000 capture events of more than 14.000 individual toads, subsuming many metamorphs. Longevity in this population exceeds the duration of our study up to now: we still encounter toads photographed in the first year. Natal dispersal appears to depend strongly on habitat structure: Toads metamorphosing on moist meadows often stay close to their birth sites, whereas those originating in temporary pools with bare surroundings usually move farther away. Since the start of our investigation, average temperatures have been rising. We discuss possible effects of climate change, which may be due to longer annual activity periods, on life-history traits in this population.

**OSCAR DESERVING PERFORMANCE: DEATH-FEIGNING IN DICE SNAKES**

ANA GOLUBOVIĆ

Faculty of Biology, University of Belgrade, Belgrade, Serbia

MARKO ANĐELKOVIĆ

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

SLAĐANA GVOZDENOVIĆ

Montenegrin Ecologists’ Society, Podgorica, Montenegro

GORAN ŠUKALO

Faculty of Natural Sciences and Mathematics, University of Banja Luka, Banja Luka, Bosnia and Herzegovina

LJILJANA TOMOVIĆ

Faculty of Biology, University of Belgrade, Belgrade, Serbia

XAVIER BONNET

Centre National de la Recherche Scientifique, Chizé, France

Dice snakes (*Natrix tessellata*) display an extensive repertoire of antipredator behaviors. They flee when they detect an attacker, and if captured, they vigorously struggle to evade. They spin their body, while spraying the foe with a malodorous mixture of cloacal content and cloacal gland secretions. Ultimately, dice snakes can switch to death-feigning (DF, thanatosis), i.e. tonic immobility in inverted position; usually opening their mouth, protruding their tongue, and sometimes producing bubbles with blood and saliva. However DF is a dangerous option since vital organs, like the liver, are exposed to the predator. We examined antipredator behaviors triggered by human observers, comparing capture-induced DF among five populations. DF prevalence ranged from 6.4% to 31.5%, probably due to different local predator pressures. We found no effects of dorsal coloration pattern or sex on DF frequencies. In Golem Grad population, a large sample (N=1,909) showed that DF frequency increased with body size. This might indicate to protective effect of DF from gape-limited predators. DF was three times less frequent in gravid than in non-gravid females. In another experiment we compared duration of DF in water and on land, after half a minute long standardized manipulation. Snakes released in container with water significantly decreased DF duration comparing to those released on land. Overall, different factors influence the occurrence and duration of DF, suggesting that this “desperate” attempt is under selection.



**COSTLY AVOIDANCE: ANURAN SPATIAL ANTIPREDATOR RESPONSES  
AGAINST FISH AT THE OVIPOSITION AND LARVAL STAGES**

JANUSZ KLOSKOWSKI

Institute of Zoology, Poznań University of Life Sciences, Poznań, Poland

MAREK NIEOCZYM

Department of Zoology and Animal Ecology, University of Life Sciences, Lublin, Poland

ROBERT STRYJECKI

Department of Zoology and Animal Ecology, University of Life Sciences, Lublin, Poland

Both reproducing amphibian females and tadpoles are known to avoid fish, important predators of tadpoles, but this behaviour may be associated with other risk factors. When choosing oviposition sites, amphibians trade-off between the risks of offspring mortality to fish in permanent habitats and premature desiccation of less permanent habitats. In habitats containing fish, tadpoles trade-off between the risks of fish and insect predation. We investigated the spatial antipredator responses against fish in large-scale, whole-system natural experiments. When three oviposition habitat types were available in close proximity – permanent fish-containing ponds and desiccation-prone pools either without fish but containing fish odour or without any fish cues ('fishless'), anuran spawn was virtually absent from the pools combining both risks. *Rana* frog spawn was present in all fishless pools, but also relatively frequent in fish-containing ponds. The fish-tolerant *Bufo bufo* preferred oviposition in fish-containing ponds. *Pelobates fuscus*, *Bufo viridis* and *Hyla arborea* oviposited virtually only in fishless pools. At the larval stage, we examined distributions of anuran tadpoles and their insect predators (anisopteran larvae and dytiscids) between microhabitats with contrasting complexity in ponds that were either kept fishless or stocked with fish. The densities of insect predators and tadpoles, including *B. bufo*, showed positive interactions between fish presence and the use of complex littoral habitat providing shelter from fish. This indicates that avoidance of fish, shared with insect predators, may put tadpoles at greater risk of predation by insects. Our results suggest costs of fish avoidance at various stages of anuran reproduction.

**TO STICK OR NOT TO STICK: FUNCTIONAL ECOMORPHOLOGY OF LIMBS AND TAIL AND THEIR SIGNIFICANCE FOR LOCOMOTION IN THREE SPECIES OF EUBLEPHARID GECKOS (SQUAMATA: SAURIA: EUBLEPHARIDAE)**

THORE KOPPETSCH

Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany

WOLFGANG BÖHME

Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany

SEBASTIAN BÜSSE

Zoological Institute, Kiel University, Am Botanischen Garten 9, 24118 Kiel, Germany

STANISLAV N. GORB

Zoological Institute, Kiel University, Am Botanischen Garten 9, 24118 Kiel, Germany

While the morphology of the adhesive system of geckos has been investigated predominantly in a single species, the tokay (*Gekko gecko*), there exists a lack of knowledge concerning a broad diversity of gecko species. Here we focus on the Asian representatives of eublepharid geckos, as a closely related group within Gekkota, and study ecomorphological adaptations of both adhesive surfaces of toes and tails and the morphology of adhesion-supporting bone structures in three species of Eublepharidae. Surface microstructures are described by using SEM microscopy. Bony structures present in limbs and tail are compared by using 3D analysis based on micro-CT data. The presence of adhesive structures in arboreal representatives of Eublepharidae and the reduction of setae in ground-dwelling species are discussed. A clear definition of the term 'seta' taking into account morphological and developmental aspects is provided. Apart from a comparative consideration of ecomorphological specialisations of limbs, adaptations for lateral convolving of the prehensile tail found in *Aeluroscalabotes felinus* are discussed. Correlations between the life style of the respective species and their morphology of certain osseous structures in limbs and tail are shown and the significance for locomotion is outlined.

## **PROGENESIS AS AN INTRINSIC FACTOR OF ECOLOGICAL OPPORTUNITY IN NEWTS**

**BENJAMIN LEJEUNE**

Laboratory of Fish and Amphibian Ethology & Laboratory of Oceanology, Freshwater and Oceanic science Unit of reSearch (FOCUS), University of Liège, Belgium

**LUCIE BISSEY**

Laboratory of Fish and Amphibian Ethology, Freshwater and Oceanic science Unit of reSearch (FOCUS), University of Liège, Belgium

**ALEXIA EMILIA DIDASKALOU**

Laboratory of Fish and Amphibian Ethology, Freshwater and Oceanic science Unit of reSearch (FOCUS), University of Liège, Belgium

**NICOLAS STURARO**

Laboratory of Oceanology, Freshwater and Oceanic science Unit of reSearch (FOCUS), University of Liège, Belgium

**GILLES LEPOINT**

Laboratory of Oceanology, Freshwater and Oceanic science Unit of reSearch (FOCUS), University of Liège, Belgium

**MATHIEU DENOËL**

Laboratory of Fish and Amphibian Ethology, Freshwater and Oceanic science Unit of reSearch (FOCUS), University of Liège, Belgium

Ecological opportunity plays an important role in the persistence of polyphenisms by allowing for niche differentiation. It is generally defined as the existence of ‘vacant niches’ that become available when resource diversity increases or following ecological release. Yet, beyond extrinsic factors of ecological opportunity, few authors consider the potential role of developmental plasticity itself as a factor able to provide the ability, *hitherto* unavailable, to exploit available resources. Here, we studied pond living facultatively paedomorphic newts that depict a progenetic pathway to paedomorphosis (i.e. earlier offset inducing the acquisition of sexual maturity at a smaller body size than metamorphs). Facultative paedomorphosis has been shown to promote niche expansion towards underused resources in spatially heterogeneous habitats such as deep lakes. In ponds, we hypothesized that, despite low ecological opportunity associated to ecosystem size, progenesis could promote niche differentiation intrinsically, via body size reduction. We used stable isotopes and stomach contents to assess trophic niche use of the two phenotypes in relation to body size in ponds of varying dimensions. We show that not only did paedomorphs occupy a different trophic niche in all populations, corresponding to an expansion towards resources underused by metamorphs, but the smaller they were due to progenesis, the more different they were from metamorphs in terms of trophic ecology. These results suggest that beyond generally recognized fitness advantages of progenetic development, this process may also bring immediate ecological advantage by intrinsically generating ecological opportunity, allowing for the persistence of this polyphenism in spatially low heterogeneous habitats.

**CAN THE ELTONIAN NICHE BE PREDICTED? A TEST WITH SARDINIAN CAVE SALAMANDERS**

ENRICO LUNGI

Universität Trier Fachbereich VI Raum-und Umweltwissenschaften Biogeographie, Trier, Germany  
Museo di Storia Naturale dell'Università degli Studi di Firenze, Sezione di Zoologia "La Specola", Firenze, Italy  
Natural Oasis, Prato, Italy

Institute of Zoology, Chinese Academy of Sciences, Beijing, China

FABIO CIANFERONI

Istituto di Ricerca sugli Ecosistemi Terrestri, Consiglio Nazionale delle Ricerche, Monterotondo (Roma), Italy  
Museo di Storia Naturale dell'Università degli Studi di Firenze, Sezione di Zoologia "La Specola", Firenze, Italy

FILIPPO CECCOLINI

Museo di Storia Naturale dell'Università degli Studi di Firenze, Sezione di Zoologia "La Specola", Firenze, Italy

RAOUL MANENTI

Dipartimento di Scienze e Politiche ambientali, Università degli Studi di Milano, Milano, Italy

MICHAEL VEITH

Universität Trier Fachbereich VI Raum-und Umweltwissenschaften Biogeographie, Trier, Germany

CLAUDIA CORTI

Museo di Storia Naturale dell'Università degli Studi di Firenze, Sezione di Zoologia "La Specola", Firenze, Italy

GENTILE FRANCESCO FICETOLA

Dipartimento di Scienze e Politiche ambientali, Università degli Studi di Milano, Milano, Italy

University Grenoble Alpes, CNRS, Laboratoire d'Écologie Alpine (LECA), Grenoble, France

GIORGIO MANCINELLI

Dipartimento di Scienze e Tecnologie Biologiche e Ambientali (DiSTeBA),

Università del Salento, Lecce, Italy

Individual diet specialisation (IS) has significant ecological and evolutionary implications, yet its causes are still debated. We tested whether the degree of individual diet specialisation in five species of European cave salamanders (genus *Hydromantes*) can be predicted by the bioclimatic and topographic features of the sites where the populations live. We analysed the stomach contents of 395 individuals belonging to eight allopatric populations from all the five *Hydromantes* species living in Sardinia (Italy). The degree of individual diet specialisation increased with the populations' total niche width, with a slope significantly steeper than that obtained by a null model. Furthermore, IS variation across multiple salamander populations was determined by bioclimatic variables, being highest in sites with more precipitation and high vegetation index. These results indicate that individual diet specialisation in populations may be influenced by local environmental conditions, either directly, via changes in ecological opportunity, or indirectly, via effects on physiological or metabolic conditions. Climatic variables are generally recognized to influence the salamanders' Grinnellian niche, but they are also successful in predicting trophic strategy at individual and population level, i.e., their Eltonian niche.

**PREDATION ON THE ENDANGERED HUNGARIAN MEADOW VIPER (*Vipera ursinii rakosiensis*) BY BADGER (*Meles meles*) AND FOX (*Vulpes vulpes*)**

ATTILA MÓRÉ

Institute for Wildlife Conservation, Szent István University

EDVÁRD MIZSEI

Hungarian Academy of Sciences, Centre for Ecological Research, Debrecen, Hungary

Hungarian meadow viper (*Vipera ursinii rakosiensis*) is a globally endangered reptile with a few sub-populations remained after habitat loss and fragmentation. Now all habitats are protected by law in Hungary and significant conservation effort have been implemented by habitat reconstruction, development and ex-situ breeding and reintroductions, but the abundance is still very low and the impact of conservation interventions are nearly immeasurable according to densities. Hypotheses have been raised, as the predation pressure is the main factor influencing viper abundance. Here we analysed the diet of potential mammalian predators (Badger and Fox) at a Hungarian meadow viper habitat, and found high prevalence of viper remains in the processed faecal samples, and estimated a high number of preyed vipers within half season in a single site. Assuming that every faeces with viper remains are independent cases of preying only one viper individual, our result suggests in the study time at the study site at least 46 Hungarian meadow viper was preyed by Badgers, and 10 by Foxes. Effective predator control is recommended to support habitat developments and reintroduction.

**CLIMATE CHANGE AND LOCAL ADAPTATION: CHALLENGES OF A LIZARD  
WHOSE SEX IS DETERMINED BY ENVIRONMENTAL TEMPERATURE**

CATARINA RATO

CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO,  
Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando  
Quintas, Nº 7, 4485-661 Vairão, Vila do Conde, Portugal

GABRIEL MOCHALES RIAÑO

CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO,  
Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando  
Quintas, Nº 7, 4485-661 Vairão, Vila do Conde, Portugal

VALÉRIA MARQUES

CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO,  
Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando  
Quintas, Nº 7, 4485-661 Vairão, Vila do Conde, Portugal

IOLANDA SILVA-ROCHA

CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO,  
Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando  
Quintas, Nº 7, 4485-661 Vairão, Vila do Conde, Portugal

JOHN ARCHER

CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO,  
Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando  
Quintas, Nº 7, 4485-661 Vairão, Vila do Conde, Portugal

MIGUEL A. CARRETERO

CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO,  
Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando  
Quintas, Nº 7, 4485-661 Vairão, Vila do Conde, Portugal

Little is known about the physiological and molecular processes that drive temperature-dependent sex determination (TSD) in reptiles, as well as its significance in terms of adaptation. Moreover, there is an absence of studies on European TSD reptiles, a research field already mastered by Australia and the USA.

Using as model the only European squamate with TSD (*Tarentola mauritanica*), this project integrates methodologies from ecology, genomics and physiology to decipher the mechanisms and adaptation dynamics of TSD within the same species. Preliminary data, indicate that warmer temperatures originate females and that males are determined by cooler incubation temperatures. This gecko has a wide geographic distribution, encompassing a variety of thermal regimes. Hence, as a TSD and widespread species, *T. mauritanica* represents the perfect model to study adaptation, and ultimately how populations will cope towards climate change, a transformation of the environment that is expected to seriously disturb the sex-ratio and population dynamics of taxa, especially of TSD species. To understand how this species maintains its sex-ratios, despite wide variation in climate among populations, a mechanistic understanding of maternal nest-site choice, pivotal temperature and adult sex-ratio is needed. To achieve this, we use four Iberian populations experiencing distinct climatic conditions. It is expected that populations will match incubation settings with local climatic conditions, through population-specific adjustments of maternal nest-site choice. Additionally, the effects of increased temperature and thermal variance, predicted to occur in a climate change scenario, during incubation are tested, as these are predicted to cause a sex-ratio bias and population disruption.

Finally, this project intends to discover the candidate genes involved in TSD by analysing patterns of gene expression.

## CLUTCH CANNIBALISM BY ADULT POISON FROGS

EVA RINGLER

University of Veterinary Medicine, Vienna, Vienna, Austria

SANDRA SPRING

University of Vienna, Vienna, Austria

MARION LEHNER

University of Vienna, Vienna, Austria

STEFFEN WEINLEIN

University of Vienna, Vienna, Austria

KRISTINA B. BECK

University of Vienna, Vienna, Austria

LUDWIG HUBER

University of Veterinary Medicine, Vienna, Vienna, Austria

MAX RINGLER

University of Vienna, Vienna, Austria

Adults may act supportive, neutral, or hostile towards conspecific progeny. We investigated corresponding behavioural responses in the poison frog *Allobates femoralis*, a species with predominant male care. In several experiments we controlled the territorial and reproductive state of adult males and females, the presence of partners, and the location and parentage of clutches. Males followed the simple rule ‘care for any clutch inside my territory’, but immediately switched from care to cannibalism when they encountered clutches outside their territory. Females remembered the exact locations of their oviposition sites and provided care only for clutches found precisely at these sites. Females preyed upon unrelated clutches only when no father was simultaneously present and in the absence of preceding own oviposition. The sex-specific parental care and cannibalism in *A. femoralis* reflects respective differences in costs of offspring confusion between the sexes, resulting from differential spatial and reproductive behaviour, and provides new insights into determinants of non-parental infanticide.

**COMPOST DWELLERS: STRATEGIC FEEDING AND USE OF INDIRECT CUES  
IN ACTIVELY FORAGING POISON FROGS**

MAX RINGLER

University of Vienna – Dept. of Integrative Zoology & Dept. of Cognitive Biology, Vienna, Austria

ANDRIUS PAŠUKONIS

Stanford University – Dept. of Biology, Stanford, U.S.A.

EVA RINGLER

University of Veterinary Medicine Vienna – Messerli Research institute of Comparative Cognition; University of Vienna –  
Dept. of Integrative Zoology & Dept. of Cognitive Biology, Vienna, Austria

While amphibians are mostly described as opportunistic feeders, Neotropical poison frogs (Dendrobatidae) exhibit active foraging, supporting the fast metabolism which enables their elaborate social and spatial behaviour. During anecdotal observations we saw female Brilliant-thighed Poison Frogs (*Allobates femoralis*) aggregating near rotting fruits (*Clusia grandifolia*) to feed on insects and their larvae, which had been attracted by the smell. Following up, we conducted a resource supplementation experiment over three years in an experimental population of *A. femoralis*, where we placed feeding and control sites, using fruits and kitchen scraps to attract prey insects. While we observed only a few males at the resource sites, we encountered up to half of the female population feeding on attracted insects. Females were usually seen at feeding sites for several days before they returned to their regular home ranges, and sometimes made repeated use after having mated and produced a clutch. This suggests that they were using the provided resources strategically beyond immediate metabolic needs, to boost egg production. Females were arriving from up to 40 meters from their usual centres of activity, indicating that they were not directly localizing prey items, but rather were attracted by the smell of the rotting fruits as an indirect cue.



**PERCH HIGHER AND BE QUIETER: ACOUSTIC ADAPTATION AND SOUND RADIATION PATTERNS IN *Allobates femoralis***

CAMILO RODRÍGUEZ

Department of Cognitive Biology, University of Vienna, Vienna, Austria

ADOLFO AMÉZQUITA

Department of Biological Sciences, University of Los Andes, Bogotá, Colombia

MAX RINGLER

Department of Integrative Zoology, University of Vienna, Vienna, Austria

ANDRIUS PAŠUKONIS

Department of Biology, Stanford University, Stanford, USA

WALTER HÖDL

Department of Integrative Zoology, University of Vienna, Vienna, Austria

The transmission of calls in frog males can be affected by many acoustic and environmental characteristics, which can influence the detection and decoding of the signal by the receiver. Calling-perch height has a strong influence on sound propagation but how frogs optimize their calling behaviour in this context is poorly understood. Our aim was to study how the use of certain habitat features influences acoustic communication in the brilliant-thighed poison frog *Allobates femoralis* by evaluating the relationship between calling-perch height, nearest-neighbour distance, and sound-pressure level. We found that frogs calling at higher perches produce softer calls, but still have larger inter-individual distances. In accompanying signal propagation experiments, low perch height differences did not affect the received sound-pressure levels. Hence, frogs perching higher keep longer sound propagation and larger inter-individual distances despite low calling amplitude. Additionally, we demonstrate that the radiation pattern of the advertisement call is nearly omnidirectional. Our results suggest the existence of an adaptive calling behaviour that reduces energy expenditure by calling at higher perches.

**TROPHIC ECOLOGY OF ALPINE SALAMANDER (*Salamandra atra atra*,  
LAURENTI, 1768)**

LUCA RONER

MUSE, Museo delle Scienze, Sezione di Zoologia dei Vertebrati, Corso del Lavoro e della Scienza 3, 38122, Trento, Italy

PAOLO PEDRINI

MUSE, Museo delle Scienze, Sezione di Zoologia dei Vertebrati, Corso del Lavoro e della Scienza 3, 38122, Trento, Italy

ANTONIO ROMANO

MUSE, Museo delle Scienze, Sezione di Zoologia dei Vertebrati, Corso del Lavoro e della Scienza 3, 38122, Trento, Italy

Consiglio Nazionale delle Ricerche, Istituto per i Sistemi Agricoli e Forestali del Mediterraneo, Via Patacca, 84, 80056

Ercolano (NA), Italy

Dietary studies on amphibians are an indispensable tool for assessing their ecological role. *Salamandra atra atra*, is the most widespread subspecies of Alpine Salamander in Europe but information on its trophic ecology are scattered and anecdotal. We studied for the first time the trophic niche and prey availability of a population from an area located in the “Paneveggio-Pale di San Martino” Natural Park. Considering that *Salamandra atra* is a typical nocturnal species, we collected food availability (with pitfalls traps) separately for diurnal and nocturnal hours. Our aims were: (i) to obtain information on the realized trophic niche; (ii) to compare total and night availability for highlighting any significant differences in potential prey composition and in determination of selectivity; (iii) to provide a direct comparison between trophic strategies considering only nocturnal preys or considering all preys. We obtained prey from 50 individuals using *stomach-flushing* technique. Trophic strategy was assessed using a modified graphical Costello method while selectivity was analysed using Relativized Electivity Index. Results showed that this salamander uses a generalized trophic strategy and trophic strategy and selectivity data indicated a preference for large sized *taxa*. The total trophic availability is significantly different from night availability. Our study provides the first data on the trophic habits of Alpine Salamander. Furthermore, we highlighted that taking in account or not the activity time of the studied taxon and its preys may lead to different interpretation of the trophic strategies.

**TROPHIC STRATEGY AND PREY AVAILABILITY OF TWO POPULATIONS OF  
*Euproctus montanus* (SAVI, 1838)**

GIACOMO ROSA

DISTAV – Università di Genova, Corso Europa 26, 16132 Genova, Italia

ANDREA COSTA

DISTAV – Università di Genova, Corso Europa 26, 16132 Genova, Italia

SEBASTIANO SALVIDIO

DISTAV – Università di Genova, Corso Europa 26, 16132 Genova, Italia

In the period May-July 2018, thanks to the Erasmus + trainsheep's project and the "Mapping and distribution" SEH grant, we carried out a research concerning eco-morphological and conservation aspects of the Corsican brook newt, *Euproctus montanus*. The present study consists in a comparison between two populations, one in the Campitellu-Bigorno Mediterranean area in the northern, and the other in a mountain stream in the Hospital forest in the southern part of Corsica. In both sites we analyzed the trophic strategies at the population and individual level, together with prey availability, and newt density. Prey abundance was similar in the two streams, while prey diversity was higher in the Mediterranean lowland site. In both sites newts' diet broadly overlapped with the available prey resources and, therefore, both populations behaved as trophic generalists. However, only in the Mediterranean lowland stream there was a significant degree of individual trophic specialization. These findings confirm the classical hypothesis of niche variation, which suggests that populations relaxed from competition or disposing of more diverse food resources may become more generalized by increasing their intra-population variation, and specifically by increasing individual specialization.

**THIRTY YEARS OF CAVE SALAMANDER POPULATION DYNAMICS... AND BEYOND**

SEBASTIANO SALVIDIO

DISTAV, University of Genova, Italy; Gruppo Speleologico "A. Issel", Busalla (GE), Italy

FABRIZIO ONETO

DISTAV, University of Genova, Italy; Gruppo Speleologico "A. Issel", Busalla (GE), Italy

MAURO VALERIO PASTORINO

Gruppo Speleologico "A. Issel", Busalla (GE), Italy

Long-term studies are needed to better understand ecological processes, such as population fluctuations, interactions among populations and biological effects of climate. In this framework, since 1990, we surveyed a population of the cave salamander *Speleomantes strinatii* (Aellen, 1958) living inside the Biospeleological Station of S. Bartolomeo di Besolagno (Province of Genova, NW Italy). In this subterranean site, salamanders display a regular pattern of seasonal activity that peaks during summer, when the outside weather is hot and dry. Each year in July, salamanders active on the cave walls were counted to obtain an index of relative population abundance. In addition, starting in 1996, a three-occasions removal experiment was conducted to estimate absolute population size. The count index and the estimated population size showed comparable levels of variability and similar temporal trends, suggesting that the count index could be used as an informative proxy for the population pattern of variation. The salamander time series was then analyzed within the Box-Jenkins' (1976) statistical framework. Autocorrelation function and autoregressive moving average (ARMA) analyses were used to model salamander' population dynamics. Time series analysis suggested that, during the 30-year study period, the salamander population dynamics was stationary and predictable with a global Lyapunov exponent  $\lambda < 0$ . Furthermore, the salamanders' time series displayed evidence of statistical periodicity, a pattern that has been rarely described in populations living in subterranean environments.

## **SEX-PHEROMONES IN NEOTROPICAL FROGS: COMMUNICATION BEYOND CALLING**

LISA M. SCHULTE

Department of Wildlife-/Zoo-Animal-Biology and Systematics, Faculty of Biological Sciences, Goethe University Frankfurt; Frankfurt, Germany

FRANKY BOSSUYT

Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel, Brussels, Belgium

Chemical signaling is an important component of the courtship display of many aquatic and terrestrial salamanders. Most known pheromones are peptides or proteins which are expressed and secreted from species-specific male breeding glands during the reproductive season to attract females or to stimulate their mating behaviours. Because anurans (i.e. frogs and toads) spend a lot of energy in acoustic and visual signaling, chemical communication during courtship has received much less attention in this amphibian order. However, anurans also have a wide diversity of sexually dimorphic glands, which due to their association with peculiar breeding behaviours and their histological similarity to salamander glands have been suggested to contain courtship pheromones as well. Because of this growing awareness that chemosignals in frogs may be more common than currently appreciated, we collected different hylid species during their reproductive seasons, covering a diverse selection of breeding gland types and combined histology with transcriptomics, proteomics and molecular phylogenetics to search for pheromone candidates. Our analyses show highly different expression patterns for different types of glands, but indicate that several of them may be associated with courtship behaviours in certain species. Our study thus further supports a role of breeding glands in chemical communication during anuran courtship.

**FLAUNTING MALES AND CAUTIOUS FEMALES – SEASONAL VARIATION IN BEHAVIOUR AND HABITAT USE OF THE FIRE SALAMANDER (*Salamandra salamandra*) IN A TEMPERATE NORTH-WESTERN EUROPEAN FOREST**

JEROEN SPEYBROECK

Research Institute for Nature and Forest Affiliation, Brussels, Belgium

While certain environmental drivers are said to correlate to the level of above-ground activity of fire salamanders *Salamandra salamandra*, long-term monitoring data to corroborate this is mostly lacking. This is particularly true for lowland populations outside the continental part of the species range, where a significant part of the yearly reproductive cycle takes part during the relatively mild winter months. Particularly under these more benign conditions, little is known on the seasonal variation in reproductive behaviour and habitat use, and on how these may vary with sex and age.

From 2010 until 2018, 223 standardised counts of active salamanders were conducted along a fixed transect in a forest patch in Flanders, Belgium. From 2014 until 2018 (189 counts), individual activity and habitat use were noted as categorical variables, as well as sex and body size. High numbers of count events and of collected observations (n= 14288, of which 12736 with individual data) allows for modelling of total activity, with humidity and air temperature as main drivers. Activity and habitat use show distinct differences between sexes and throughout the year, with males being more prone to position themselves at the base of trees, as well as at the more exposed forest edge, and with both sexes displaying clear seasonal variation. The observed patterns shed new light on demographic and seasonal variation in activity, behaviour and habitat use, and may offer guidelines for forest management.

**POPULATION CHARACTERISTICS OF THE NOSE-HORNED VIPER (*Vipera ammodytes*) ON GOLEM GRAD ISLAND (NORTH MACEDONIA)**

LJILJANA TOMOVIĆ

Faculty of Biology, University of Belgrade, Belgrade, Serbia

DRAGAN ARSOVSKI

Macedonian Ecological Society, Skopje, Republic of North Macedonia

ANA GOLUBOVIĆ

Faculty of Biology, University of Belgrade, Belgrade, Serbia

SONJA NIKOLIĆ

Faculty of Biology, University of Belgrade, Belgrade, Serbia

MARKO ANĐELKOVIĆ

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

BOGOLJUB STERIJOVSKI

Macedonian Ecological Society, Skopje, Republic of North Macedonia

RASTKO AJTIĆ

Institute for Nature Conservation of Serbia, Belgrade, Serbia

JELKA CRNOBRNJA ISAILOVIĆ

Faculty of Natural Sciences, University of Niš, Niš, Serbia

XAVIER BONNET

Centre National de la Recherche Scientifique, Chizé, France

The nose-horned viper (*Vipera ammodytes*) is the least studied species of European true vipers, regarding its population ecology and reproductive biology. We ran capture-recapture (CR) studies on this species during 12 years (2007-2018) on Golem Grad Island (Prespa Lake, North Macedonia). We captured 501 vipers (304 adults, 161 subadults, 36 newborns) and recaptured 240 individuals enabling us to estimate demographic traits. Females larger than 35.0 cm of snout-vent-length [SVL] (the smallest undoubtedly gravid female) and males larger than 37.0 cm SVL (the smallest male observed while mating) were considered adults. The largest captured individual was 66 cm long, indicating insular dwarfism. Fecundity data were gathered in the field (palpation) and from females collected for controlled parturition in order to obtain size at birth and define newborn size (up to 18.0 cm SVL). Intermediate sized snakes were considered subadults. Modelling of subadult and adult CR data implied sex- and age-specific capture probabilities (females and subadults being on the lower end); mean annual survival probabilities were in favour of the females (78% vs. 71%). Total population size was estimated at 2345 ( $s=293.9$ ) individuals, indicating the highest reported density for this species (130 ind/ha,  $s=16.4$ ). Adult sex-ratio was 1,4 (male/female) and the proportion of reproductive females ranged from 44% to 74%, suggesting a biennial cycle. Litter size varied from two to nine (average 4.5); this is particularly low for this species. Diet changed with ontogeny – juveniles fed mainly on *Scolopendra cingulata* and lizards, while adults mainly preyed on lizards and dice snakes.

**MECHANISMS FOR COLOR CONVERGENCE IN A MIMETIC RADIATION OF  
POISON DART FROGS**

EVAN TWOMEY

Vrije Universiteit Brussel, Brussels, Belgium

MORGAN KAIN

McMaster University, Hamilton, Ontario, Canada

MYRIAM CLAEYS

Ghent University, Ghent, Belgium

KYLE SUMMERS

East Carolina University, Greenville, North Carolina, USA

SANTIAGO CASTROVIEJO-FISHER

Pontificia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Brazil

INES VAN BOCXLAER

Vrije Universiteit Brussel, Brussels, Belgium

In animals, bright colors often evolve to mimic other species when a resemblance is selectively favored. The poison dart frog *Ranitomeya imitator* has four mimetic color morphs, each resembling a sympatric species, with colors ranging from green to orange. Understanding the mechanisms that produce such diverse colors can yield insights into how a single species is able to mimic several distinct model species. We used reflectance spectrometry, skin pigment analysis, electron microscopy, and color modeling to investigate the proximate mechanisms of color production in poison dart frogs, focusing on the *R. imitator* mimicry system. We found that color is largely controlled by the structural color produced by the iridophores, but depends crucially on interactions with pigments. Compared to each of the model species which it resembles, *R. imitator* displays greater variation in both structural and pigmentary mechanisms, which may have facilitated phenotypic divergence in this species. Analyses of non-mimetic dendrobatids in other genera demonstrate that these mechanisms are widespread within the family, and that poison dart frogs share a complex physiological “color palette” that can produce diverse and highly reflective colors.



**INVASIVE SUBTROPICAL TOADS ALLOCATE MORE RESOURCES TO GROWTH AND MAINTENANCE OVER REPRODUCTION AND STORAGE IN A MEDITERRANEAN ENVIRONMENT**

GIOVANNI VIMERCATI

Department of Biology, University of Fribourg, Fribourg, Switzerland

SARA J. DAVIES

Centre for Invasion Biology, Department of Botany & Zoology, Stellenbosch University, Stellenbosch, South Africa

JOHN MEASEY

Centre for Invasion Biology, Department of Botany & Zoology, Stellenbosch University, Stellenbosch, South Africa

Amphibians living in cold and seasonal environments allocate more resources to growth, maintenance and storage than do conspecifics from warmer and less seasonal environments. This sustained resource allocation may be obtained at the expense of reproduction, especially when low conditions of temperature and rainfall restrict breeding season length. Invasive populations act as experiments to explore how resources are allocated in novel environments. We studied the guttural toad *Sclerophrys gutturalis*, a synanthropic species which naturally inhabits subtropical areas of central and southern Africa. Guttural toads were introduced in the early 2000s to Cape Town, where they rapidly became invasive. Since Cape Town experiences a mediterranean climate, the species has been exposed to an environment that is cooler and presents a different rainfall pattern from that of the native range. We targeted the Cape Town invasive population and a native source population from Durban (South Africa). After dissection, lean structural mass (bones and muscles), gonadal mass, liver mass and body fat % were measured in 161 native and invasive animals sampled at the beginning and the end of the breeding season. As expected, male and female toads from the invaded range allocate more resources to growth and maintenance than their native counterparts, whereas invasive female toads direct fewer resources to reproduction than native ones. Unexpectedly, energy storage of guttural toads does not consistently differ between invaded and native ranges. Such allocation shift may be a response to the low temperature, reduced rainfall and heightened seasonality encountered by the invasive population.

## SEX-BIASED DISPERSAL IN THE ASP VIPER

VALERIE ZWAHLEN

Department of Environmental Sciences, Section of Conservation Biology, University of Basel, Basel, Switzerland

SILVIA NANNI-GESER

Department of Environmental Sciences, Section of Conservation Biology, University of Basel, Basel, Switzerland

LAURA KAISER

Department of Environmental Sciences, Section of Conservation Biology, University of Basel, Basel, Switzerland

JOAQUIM GOLAY

Hintermann & Weber SA, Montreux, Switzerland

SYLVAIN DUBEY

Department of Ecology and Evolution, Biophore Building, University of Lausanne, Lausanne, Switzerland

Hintermann & Weber SA, Montreux, Switzerland

SYLVAIN URSENBACHER

Department of Environmental Sciences, Section of Conservation Biology, University of Basel, Basel, Switzerland

info fauna – CSCF & karch, Neuchâtel, Switzerland

Sex-biased dispersal is the consequence of differences in costs and benefits of dispersal between sexes. This phenomenon is essential to maintain genetic diversity within and among populations. Several studies demonstrated male-biased dispersal in mammals and female-biased dispersal in birds. In reptiles, male-biased dispersal seems to be prevalent, but only a few studies were conducted in this group until now. Moreover, most of these studies considered only a single location, although one study demonstrated distinct patterns of sex-biased dispersal between populations of different locations. We investigated sex-biased dispersal in the Asp viper (*Vipera aspis*) in four locations in Switzerland using microsatellite markers. We hypothesised that males disperse farther, whereby females are more spatially autocorrelated and show a stronger isolation by distance than males. In three of the four investigated locations, a trend for male-biased dispersal was detected, while in one location female-biased dispersal was found. A possible explanation for this difference is the higher level of fragmentation of the latter location. Our study demonstrated the importance to test for sex-biased dispersal in different habitats and that general conclusions about patterns of sex-biased dispersal should be drawn with caution if studies are conducted in a single location.







## Session: Evolutionary biology and phylogeny

**EVOLUTIONARY CONSEQUENCES OF ISOLATION BY ENVIRONMENT IN  
TWO ECOMORPHOLOGICALLY DISTINCT SALAMANDERS, *Salamandra  
salamandra bejarae* AND *S. s. almanzoris***

BERNARDO ANTUNES

Institute of Environmental Sciences. Jagiellonian University, Kraków, Poland

GUILLERMO VELO-ANTÓN

CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto, Vairão, Portugal

DAVID BUCKLEY

Universidad Autónoma de Madrid, Madrid, Spain

RICARDO J. PEREIRA

Ludwig-Maximilians Universität, München, Germany

IÑIGO MARTÍNEZ-SOLANO

Departamento de Biodiversidad y Biología Evolutiva. Museo Nacional de Ciencias Naturales MNCN-CSIC, Madrid, Spain

Landscape features can influence gene flow, and by extension patterns of genetic variation, in two inherently distinct ways: through geographical isolation (usually tested by isolation by distance/resistance [IBD/IBR]) and through ecological isolation (usually associated with isolation by environment [IBE]). The evolutionary consequences of these processes might differ substantially. Generally, both types of isolation are expected to promote speciation, although geographical isolation does not necessarily imply ecological speciation – a necessary process for the emergence of taxa with specific morphology/niche associations, or ecomorphs. In central Iberia, two distinct fire salamander ecomorphs - *Salamandra salamandra bejarae* and *S. s. almanzoris* - interact along a steep altitudinal gradient. The existence of such ecomorphs has been indirectly linked to processes of ecological isolation (e.g. natural or sexual selection against immigrants). Here we use a hierarchical landscape genetics approach to test for IBE, IBD and IBR at different hierarchical levels of genetic organization within and between ecomorphs. Our results show that the role of IBE differs considerably at different hierarchical levels and between the different ecomorph ranges. In particular, we found a stronger role for IBE in the part of the range where both ecomorphs are present, whereas geographical isolation alone was enough to explain gene flow patterns in areas where only one ecomorph is present. Overall, our study shows that isolation by environment plays a central role in shaping patterns of genetic structure and admixture in *S. salamandra* in central Iberia, potentially associated with incipient past ecological speciation processes.

**SYSTEMATICS AND DIVERSIFICATION OF THE GECKOS OF THE GENUS  
*Pristurus***

SALVADOR CARRANZA

Institute of Evolutionary Biology, Barcelona, Spain

MARC SIMÓ-RIUDALBAS

Institute of Evolutionary Biology, Barcelona, Spain

HÉCTOR TEJERO-CICUÉNDEZ

Institute of Evolutionary Biology, Barcelona, Spain

KARIN TAMAR

Institute of Evolutionary Biology, Barcelona, Spain

TOMÁŠ MAZUCH

Independent Researcher, Dříteč, Czech Republic

PEDRO TARROSO

CIBIO/InBio, Centro de Investigação em Biodiversidade e Recursos Genéticos, Laboratório Associado, Universidade do Porto, Vairão, Portugal

BERNAT BURRIEL-CARRANZA

Institute of Evolutionary Biology, Barcelona, Spain

THEODORE PAPPENFUSS

Department of Integrative Biology, Museum of Vertebrate Zoology, University of California, Berkeley, USA

The sphaerodactylid geckos of the genus *Pristurus*, also known as Semaphore geckos, comprise 25 described species, characterized by being mostly diurnal, heliothermic and by signaling each other by waving their tails. These features are unusual among geckos, which are mainly nocturnal, active at comparatively low temperatures and communicate predominantly by vocalization or by chemical cues. In fact, some *Pristurus* species behave more like desert agamids than typical geckos. Most of the species are distributed mainly across Arabia, the Socotra Archipelago and northeast Africa, with one isolated species in Mauritania, constituting a 4,700 km range extension, and another one extending to the west coast of Iran. Despite the interest of *Pristurus* from a biogeographical, evolutionary, ecological and behavioral point of view, the genus has been relatively neglected compared to other gecko genera from the same region. Preliminary studies indicate that, even though the different species present high levels of habitat, climatic and morphological differentiation, there are many morphologically cryptic deep lineages that could nearly double the number of currently described species. Moreover, contrary to what is expected, continental species present higher levels of phenotypic diversification compared to insular groups, highlighting the importance of taxon-dependent factors at determining patterns of morphological diversification. In this talk we will present a comprehensive review of the systematics, biogeography and evolution of the genus *Pristurus* using molecular and morphological data and including an unprecedented sampling across its distribution range. Our results highlight, once more, the importance of reptiles in Arabian biodiversity.

**CONSERVATION GENETICS OF THE LEOPARD TORTOISE *Stigmochelys pardalis***

URBAN DAJČMAN

University Ljubljana, BIOTECHNICAL FACULTY, department of biology, Jamnikarjeva 101, 1000 Ljubljana, SLOVENIA  
Museum of Zoology, Senckenberg Dresden, A. B. Meyer Building, 01109 Dresden, GERMANY

MARGARETHA D. HOFMEYR

Chelonian Biodiversity and Conservation, Department of Biodiversity and Conservation Biology, University of the Western  
Cape, Bellville 7535, SOUTH AFRICA 3,4Unit for Environmental Sciences and Management, North-West University  
Private Bag X6001 Potchefstroom 2520, SOUTH AFRICA

MELITA VAMBERGER

Museum of Zoology, Senckenberg Dresden, A. B. Meyer Building, 01109 Dresden, GERMANY

Sub-Saharan Africa harbours the highest diversity of tortoises of which the Leopard Tortoise *Stigmochelys pardalis* is the most widespread. The species is threatened by habitat modifications, over-collection for human consumption and pet trade, road mortality and electrocution by electric fences across its range. Consequently, most Leopard tortoises are nowadays restricted to private reserves and farms, with confiscated tortoises frequently being released into reserves. This is problematic, as it has been demonstrated that the Leopard Tortoise harbours five distinct genetic mitochondrial lineages, of which three occur in the southern portion of the species' distributional range. Using microsatellite and mitochondrial markers combined, our previous study showed a pronounced south-north differentiation with a clear substructure in the north of the study area. In the present study we supplemented this existing dataset with 66 additional samples by closing sampling gaps in northern South Africa. Using 14 microsatellite loci and a country-wide sampling of 270 individuals, we could show a clear substructuring in the north with four subclusters (western, central, north-eastern and eastern distribution). Genetic diversity was low in the south and high in the north of our study region, particularly in the north-east. Basic morphological analysis shows that individuals belonging to the southern cluster tend to grow bigger than the ones from the north. With our study we established a comprehensive genetic database for South Africa and Namibia that will serve for conservation genetic purposes and facilitate the assignment of translocated Leopard Tortoises.

**SPECIATION WITH HYBRIDIZATION: THE PHYLOGEOGRAPHY OF GREEN TOADS (*Bufo*) REVISITED**

CHRISTOPHE DUFRESNES

Hintermann & Weber, Montreux, Switzerland

GLIB MAZEPA

University of Lausanne, Lausanne, Switzerland

DANIEL JABLONSKI

Cornelius University of Bratislava, Bratislava, Slovakia

SPARTAK N. LITVINCHUK

Zoological Institute of St Petersburg, Russian Academy of Sciences, St Petersburg Russia

The radiation of Palearctic green toads (*Bufo*) holds a great potential for phylogeography, speciation and notably hybrid speciation research, but present knowledge remains limited to scattered regional molecular datasets. To get an integrative and comprehensive picture on *Bufo* evolution, we conducted a multi-locus study with genome-wide data (RAD-seq) combined with an extensive compilation of mtDNA, genome size, bioacoustics, niche modelling and venom composition analyses from hundreds of populations throughout Eurasia, under a realistic molecular clock work. We discovered multiple instances of cyto-nuclear discordances, i. e. a new species that lost its mtDNA (what we coin a “super-cryptic” taxon), another that captured the mtDNA of its neighbor during post-glacial expansions, and several additional cases of widespread mitochondrial introgression, especially in the Middle East / Central Asia, emphasizing the hybridization potential of green toads and the instability of their ranges during the Quaternary. Moreover, we could accurately trace the origin of several polyploid forms, providing novel insights onto the genomic plasticity and timeframe of hybrid speciation. Last but not least, our study demonstrates how classic nuclear tools such as the ones used in previous studies (e.g. mtDNA, introns) can be misleading in phylogeography, and calls for numerous taxonomic changes in *Bufo*.



**EVOLUTIONARY LABILITY IN *HOX* CLUSTER STRUCTURE AND GENE  
EXPRESSION IN *Anolis* LIZARDS**

NATHALIE FEINER  
Lund University, Lund, Sweden

*Hox* genes orchestrate development by patterning the embryonic axis. Vertebrate *Hox* genes are arranged in four compact clusters, and the spacing between genes is assumed to be crucial for their function. The genomes of squamate reptiles are unusually rich and variable in transposable elements (TEs), and it has been suggested that TE invasion is responsible for the *Hox* cluster expansion seen in snakes and lizards. Using *de novo* TE prediction on 17 genomes of lizards and snakes, we show that TE content of *Hox* clusters are generally 50% lower than genome-wide TE levels. However, two distantly related lizards of the species-rich genus *Anolis* have *Hox* clusters with a TE content that approaches genomic levels. The age distribution of TEs in *Anolis* lizards revealed that peaks of TE activity broadly coincide with speciation events. In accordance with theoretical models of *Hox* cluster regulation, we find that *Anolis* species with many TEs in their *Hox* clusters show aberrant *Hox* gene expression patterns suggesting a functional link between TE accumulation and embryonic development. These results are consistent with the hypothesis that TEs play a role in developmental processes as well as in evolutionary diversifications.

**BIOGEOGRAPHIC HISTORY OF *Cerastes* VIPERS. A TALE FROM THE  
WORLD'S WARMEST DESERTS**

MARGARIDA BARROS

Faculty of Sciences of the University of Porto, Porto, Portugal

GUILLERMO VELO-ANTÓN

CIBIO – Research Center in Biodiversity and Genetic Resources of the University of Porto, Vairão, Portugal

SALVADOR CARRANZA

Institute of Evolutionary Biology (CSIC-UPF), Barcelona, Spain

PIERRE-ANDRÉ CROCHET

CEFE, CNRS - Université de Montpellier - Université Paul-Valéry Montpellier, Montpellier, France

LUIS GARCÍA-CARDENETE

Loja, Granada, Spain

JOSÉ C. BRITO

CIBIO – Research Center in Biodiversity and Genetic Resources of the University of Porto, Vairão, Portugal

Faculty of Sciences of the University of Porto, Porto, Portugal

FERNANDO MARTINEZ-FREIRÍA

CIBIO – Research Center in Biodiversity and Genetic Resources of the University of Porto, Vairão, Portugal

Pleistocene climatic oscillations have influenced biogeographical patterns of species worldwide. The Sahara and Arabian deserts are outstanding regions to study the influence of climate on the genetic structure and variability of species given their dynamic history, and the diverse life-history and habitat traits of taxa inhabiting such extreme regions. This study aims to address the role of Pleistocene climatic oscillations in the evolutionary histories of the three *Cerastes* viper species (Viperinae): *C. cerastes* and *C. vipera* from the Sahara Desert, and *C. gasperetti* from the Arabian Peninsula. Phylogenetic structure was inferred using Bayesian inference over sequences (68 samples, mostly covering species ranges) for one mtDNA (COI) and three nuDNA (PRLR, NT3, VIM) gene markers. Paleoclimatic models combined 318 occurrences and five climatic variables in Maxent to infer climatic suitability for current and past (mid Holocene, Last Glacial Maximum and Last Inter Glacial) events, and stability over time. Mitochondrial inferences show *C. cerastes* and *C. gasperetti* as sister taxa, while *C. vipera* as a phylogenetically more distant species. Further levels of mtDNA structure within the three species originated along the middle and late Pleistocene. Nuclear inferences, however, resulted in important discordances to mtDNA patterns. Paleoclimatic models identified warm events as major drivers of range reduction and isolation for the three species. Areas of high climatic stability across the Sahara and Arabian deserts likely acted as Pleistocene climatic refugia at the intraspecific level. This multidisciplinary approach allows to propose biogeographic scenarios for the evolution of these desert-adapted species.

**EVOLUTIONARY VARIATION IN MORPHOLOGY-PERFORMANCE  
ASSOCIATIONS IN *Podarcis* WALL LIZARDS**

VERÓNICA GOMES  
CIBIO/InBIO, Vairão, Portugal  
ANTIGONI KALIONTZOPOULOU  
CIBIO/InBIO, Vairão, Portugal

Form and function have always intrigued biologists, as they constitute a key element of the evolutionary history of organisms. Under the ecomorphological paradigm, differences in morphological traits result in differences in performance which in turn translate into variation in fitness in a given environment. Understanding how such evolutionary associations emerge at the microevolutionary level (i.e. across individuals of a species) and then scale up to shape macroevolutionary patterns (i.e. across species) has long been a challenge in evolutionary biology. In the Mediterranean Basin, wall lizards (*Podarcis spp.*) are an intriguing model system for investigating the evolutionary meaning of phenotypic traits and their relationship with performance, due to their elevated variability and their occurrence in a diversity of both natural and anthropized habitats. We examined locomotion and bite force, and several morphological traits relevant for functional performance for several species of wall lizards. We then obtained F-matrix statistics that describe the association between form and function within (intraspecific) and across species (interspecific). We also used pairwise Mantel tests to investigate the functional divergence among species and to study whether these were in accordance with clade-level phenotype-performance relationships. Mantel tests on intraspecific F-matrices suggested some degree of functional divergence among species. Our results also showed that intra- and interspecific F-matrices provide different information, where phenotype-performance relationships often do not coincide across analytical levels. Overall, our results suggest that phenotype-performance relationships may vary substantially across species and evolutionary scales.

**PHYLOGENY AND BIOGEOGRAPHY OF NARROW-MOUTHED FROGS OF THE  
GENUS *Microhyla* (AMPHIBIA: MICROHYLIDAE)**

VLADISLAV A. GORIN

Biological Department of the Lomonosov Moscow State University, Moscow, Russia

EVGENIYA N. SOLOVYEVA

Zoological Museum of the Lomonosov Moscow State University, Moscow, Russia

SURANJAN KARUNARATHNA

Nature Explorations and Education Team, Moratuwa, Sri Lanka

MAHMUDUL HASAN

Institute for Amphibian Biology, Hiroshima University, Higashihiroshima, Japan

INDRANEIL DAS

Institute of Biodiversity and Environmental Conservation, University Malaysia Sarawak, Kuching, Malaysia

NIKOLAY A. POYARKOV

Biological Department of the Lomonosov Moscow State University, Moscow, Russia

The narrow mouthed frogs of the genus *Microhyla* (Amphibia, Microhylidae) inhabit various altitudes and habitats from primary tropical forests to paddy fields in South, Southeast and East Asia. Possibly due to small size and superficial morphological similarity the taxonomy of the genus was long neglected and currently is in a state of a flux complicated with findings of lineages that cannot be related to any of described species. In this report we present the most complete phylogenetic analysis and shed light on historical biogeography and body size evolution of the genus *Microhyla*. Our work offers the most complete taxon sampling to date by including more than 90% of the diversity of the genus (39 out of 43 species). We used fragments of mtDNA genes (2598 b.p. of 12S rRNA, tRNA-Val and 16S rRNA) and nuDNA gene (720 b.p. of BDNF) to analyze 225 samples of Microhylidae. Analyses provide the most comprehensive taxonomic and gene sampling for *Microhyla* to date and reconstruct biogeographic scenario for the genus. Our analyses indicate presence of at least 6 undescribed species-level lineages of *Microhyla*. Extreme miniaturization appears in different lineages of *Microhyla* independently and we tend to associate it with ecological specialization. Our results contribute to the interpretation of diversification patterns of Southeast Asia batrachofauna and provides insights into the historical biogeography of this region. The research was supported by the Russian Science Foundation RSF, grant No. 19-14-00050.

**ARE *Podarcis* WALL LIZARDS AN ADAPTIVE RADIATION?  
JOINING PHYLOGENETIC AND PHENOTYPIC EVIDENCE**

ANTIGONI KALIONTZOPOULOU

CIBIO/InBIO, Vairão, Portugal

VERÓNICA GOMES

CIBIO/InBIO, Vairão, Portugal

INÊS FREITAS

CIBIO/InBIO, Vairão, Portugal

MIGUEL ÁNGEL CARRETERO

CIBIO/InBIO, Vairão, Portugal

CATARINA PINHO

CIBIO/InBIO, Vairão, Portugal

DANIELE SALVI

University of L'Aquila, L'Aquila, Italy

Deciphering how species and phenotypic diversity emerge is a major objective of evolutionary research, with important implications for our capacity to protect biodiversity. A powerful tool to understand how historical and contemporary processes influence the evolution of biodiversity is to combine phylogenetic inferences with information on phenotypic traits which reflect ecological responses to the environment. *Podarcis* wall lizards are a symbolic element of Mediterranean ecosystems, and their evolution is representative of the complex biogeographic and climatic history of this region. Their phylogenetic relationships have long puzzled European herpetologists, due to a basal polytomy, which may point to a quick radiation early in the history of the group. We combined a phylogenetic hypothesis inferred based on mitochondrial and nuclear genes and a series of morphological and functional traits, to test whether there are signs of an adaptive radiation in this genus of Mediterranean lizards. The cladogenetic pattern is in accordance with a scenario of a fast diversification early in the history of the genus, with the emergence of new lineages slowing down through time. However, phenotypic traits provide mixed evidence: while body size seems to have evolved under random processes, relative head size and limb length, as well as sprinting capacity, bare signs of adaptive evolution. Interestingly, though, our results do not support a scenario of an early burst of phenotypic diversification under an adaptive radiation in this group. Instead, phenotypic evolution may have rather proceeded in response to other ecological factors, such as insularity or habitat use.

**MOLECULAR PHYLOGENY OF VALENTIN'S LIZARD, *Darevskia valentini*,  
(BOETTGER, 1892) REVEALS NEW GENETIC LINEAGES AND MAY SUGGEST  
TAXONOMIC RECONSTRUCTION**

KAMİL CANDAN\*

Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir, Turkey

PANAGIOTIS KORNILIOS

Institute of Evolutionary Biology (CSIC, UPF), Barcelona, Spain

ALEXEY YANCHUKOV

Zonguldak Bülent Ecevit University, Faculty of Science and Arts, Department of Biology, Zonguldak, Turkey

YUSUF KUMLUTAŞ

Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir, Turkey

DİNÇER AYZAZ

Ege University, Faculty of Science, Department of Biology, İzmir, Turkey

DAVID TARKHNISHVILI

Ilia State University, Tbilisi, Georgia

MARINE MURTSKHVALADZE

Ilia State University, Tbilisi, Georgia

SOFIKO KURDADZE

Ilia State University, Tbilisi, Georgia

NATIA BARATELI

Ilia State University, Tbilisi, Georgia

MARIAM GABELAIA

Ilia State University, Tbilisi, Georgia

MEHMET KÜRŞAT ŞAHİN

Hacettepe University, Faculty of Science, Department of Biology, Ankara, Turkey

FERHAT MATUR

Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir, Turkey

SERKAN GÜL

Recep Tayyip Erdoğan University, Faculty of Science and Arts, Department of Biology, Rize, Turkey

ELİF YILIDIRIM CAYNAK

Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir, Turkey

FARUK ÇOLAK

Zonguldak Bülent Ecevit University, Faculty of Science and Arts, Department of Biology, Zonguldak, Turkey

ÇETİN ILGAZ

Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir, Turkey

\*Corresponding: kamil.candan@deu.edu.tr

*Darevskia valentini* is the most widely distributed species among the Caucasian rock lizards but the phylogenetic relationship among geographically distant populations is poorly known. We sequenced several mitochondrial (12S rRNA, COI and cyt b) and nuclear (MC1R and cmos) genes from 75 individual samples collected across Central and Eastern Turkey. In total, 349 bp of 12S rRNA, 615 bp of COI, 743 bp of cyt b of mtDNA and 620 bp of MC1R, 326 bp of cmos of nDNA were obtained. Additionally, 34 individuals from Georgia and Eastern Turkey were genotyped for 10 microsatellite loci. Phylogenetic analyses (ML and BI) produced trees with similar topologies including well-supported clades. One clade found in the area of Lake Van is clearly separated from others on the phylogenetic tree, and the respective population formed distinct STRUCTURE and PCA clusters in the microsatellite data. Our results may suggest that *D. valentini*, one of the most complex species in *Darevskia* genus, needs taxonomical revision at the subspecies level.

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**TOWARDS EVIDENCE-BASED SPECIES DELIMITATION IN EURASIAN VIPERS**

FERNANDO MARTÍNEZ-FREIRÍA

CIBIO – Research Center in Biodiversity and Genetic Resources of the University of Porto, Vairão, Portugal  
INÊS FREITAS

CIBIO – Research Center in Biodiversity and Genetic Resources of the University of Porto, Vairão, Portugal  
KONRAD MEBERT

Global Biology, Waldmattstr, Birr, Switzerland

IDECC, Institute of Development, Ecology, Conservation and Cooperation, Rome, Italy

OLEKSANDR ZINENKO

Museum of Nature, V. N. Karazin Kharkiv national University, Kharkiv, Ukraine

SILKE SCHWEIGER

Naturhistorisches Museum Wien, Vienna, Austria

WOLFGANG WÜSTER

MEFGL, School of Natural Sciences, Bangor University, UK

JOSÉ CARLOS BRITO

CIBIO – Research Center in Biodiversity and Genetic Resources of the University of Porto, Vairão, Portugal  
JELKA CRNOBRNJA-ISAILOVIĆ

Faculty of Sciences and Mathematics University of Niš, Serbia

Institute for biological research “S. Stanković” University of Belgrade, Serbia

BÁLINT HALPERN

MME BirdLife Hungary, Budapest, Hungary

SOUMIA FAHD

Faculté des Sciences de Tétouan, Université Abdelmalek Essaâdi, Tétouan, Morocco

XAVIER SANTOS

CIBIO – Research Center in Biodiversity and Genetic Resources of the University of Porto, Vairão, Portugal  
JUAN M. PLEGUEZUELOS

Departamento de Zoología, Facultad de Ciencias, Universidad de Granada, Granada, Spain

ULRICH JOGER

State Museum of Natural History, Braunschweig, Germany

NIKOLAY ORLOV

Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia

EDVÁRD MIZSEI

Department of Ecology, University of Debrecen, Hungary

OLIVIER LOURDAIS

Centre d'Etudes Biologiques de Chizé, CNRS, Villiers en Bois, France

MARCO A. L. ZUFFI

Museum Natural History, University of Pisa, Italy

ALEXANDRU STRUGARIU

Faculty of Biology – Research Department, “Alexandru Ioan Cuza” University of Iași, Romania

ȘTEFAN REMUS ZAMFIRESCU

Faculty of Biology – Department of Biology, “Alexandru Ioan Cuza” University of Iași, Romania

ÍÑIGO MARTÍNEZ-SOLANO

Museo Nacional de Ciencias Naturales - CSIC, Madrid, Spain

GUILLERMO VELO-ANTÓN

CIBIO – Research Center in Biodiversity and Genetic Resources of the University of Porto, Vairão, Portugal  
ANTIGONI KALIONTZOPOULOU

CIBIO – Research Center in Biodiversity and Genetic Resources of the University of Porto, Vairão, Portugal  
SYLVAIN URSENBACHER

Department of Environmental Science, Section of Conservation Biology, University of Basel, Basel, Switzerland  
Karch, Centre de coordination pour la protection des amphibiens et des reptiles de Suisse, Neuchâtel, Switzerland

The designation of taxonomic units has important implications on the way we study, understand and catalogue biodiversity, as well as on how we mobilize efforts and allocate funds to develop conservation strategies. Eurasian vipers are a monophyletic group (Serpentes, Viperinae), currently comprising four genera (*Daboia*, *Macrovipera*, *Montivipera* and *Vipera*) and up to 40 species. This group has a long and complex taxonomic history, and thus, taxonomic units have been defined and described using a wide variety of methods and criteria. Consequently, considerable controversy still surrounds the validity of some of the currently listed species. In order to advance towards a consensual and evidence-based taxonomy of Eurasian vipers, we analyzed published mitochondrial and nuclear DNA sequences for this group to identify phylogenetic relationships within currently recognized viper species. We also compiled information on external morphology to address the morphological distinctiveness of currently recognized species. Our mtDNA phylogenetic inferences show contrasting levels of divergence across the different species and some species with paraphyletic positions. The nuclear data show

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extremely low levels of genetic variation, with a wide pattern of haplotype sharing among distant species, and even among genera. Our morphological data compilation shows that most species designations rely on scalation traits that largely overlap among species of the same genus. Based on our combined assessment, we provide recommendations on the specific status of described species, and also propose further directions of research to achieve the recognition of coherent taxonomic units within Eurasian vipers.



**REVISION OF THE TAXONOMY AND PHYLOGENETIC HISTORY OF  
PALEARCTIC WATER FROGS (*Pelophylax*)**

GLIB MAZEPA

Department of Ecology and Evolution, University of Lausanne, Biophore, 1015, Switzerland  
Department of Ecology and Genetics, Evolutionary Biology, Norbyvägen 18D, 75236 Uppsala, Sweden

SPARTAK N. LITVINCHUK

Group of Genome Microevolution and Cytoecology, Institute of Cytology, Russian Academy of Sciences, Tikhoretsky Pr. 4, St. Petersburg  
194064, Russia

MACIEJ PABIJAN

Department of Comparative Anatomy, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland

DANIEL JABLONSKI

Department of Zoology, Comenius University in Bratislava, Mlynská dolina B-1, 842 15, Bratislava, Slovakia

JOCHEN B. W. WOLF

Division of Evolutionary Biology Faculty of Biology LMU Munich Grosshaderner Str. 2. 82152 Planegg-Martinsried, Germany

NICOLAS PERRIN

Department of Ecology and Evolution, University of Lausanne, Biophore, 1015, Switzerland

Water frogs of the genus *Pelophylax* represent one of the most complex groups of Palearctic amphibians, the taxonomy of which has been historically challenged by a highly conserved phenotype while molecular biogeography and phylogenetics are hampered by the fragmentary and generally non-complementary data.

Aiming to get new insight into *Pelophylax* evolutionary drama and establish a comprehensive phylogenetic framework, we generated several dozen novel mitochondrial assemblies in ensemble with multi-locus ddRAD dataset from numerous populations across the Palearctic. Our approach allowed to systematise existing, yet often non-orthologous mitochondrial data, as well as address nuclear genetic relationships within the group, bringing on the stage a number of so far overlooked water frog lineages. In particular, we compared several known phylogenetic scenarios by employing other Ranidae mitogenomes and fossil calibrations, assessed patterns of admixture in several contact zones, and investigated paraphyly in some well-established taxa. As a result, we propose new phylogenetic and taxonomic hypotheses of the *Pelophylax* to the SEH venue, which might also entail implications for amphibian conservation in Europe.

**IDENTIFICATION AND CHARACTERIZATION OF TWO TRANSPOSABLE ELEMENTS AND AN AMNIOTE ULTRA-CONSERVED ELEMENT (UCE) IN THE GENOME OF *Zootoca vivipara***

MARCELLO MEZZASALMA

Department of Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD, UK

FABIO M. GUARINO

Dipartimento di Biologia, Università di Napoli Federico II, Via Cinthia 26, 80126 Napoli, Italy

LARISSA KUPRIANOVA

Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia

GAETANO ODIERNA

Dipartimento di Biologia, Università di Napoli Federico II, Via Cinthia 26, 80126 Napoli, Italy

MARIA M. PALLOTTA

Dipartimento di Biologia, Università di Napoli Federico II, Via Cinthia 26, 80126 Napoli, Italy

AGNESE PETRACCIOLI

Dipartimento di Biologia, Università di Napoli Federico II, Via Cinthia 26, 80126 Napoli, Italy

ORFEO PICARIELLO

Dipartimento di Biologia, Università di Napoli Federico II, Via Cinthia 26, 80126 Napoli, Italy

TERESA CAPRIGLIONE

Dipartimento di Biologia, Università di Napoli Federico II, Via Cinthia 26, 80126 Napoli, Italy

We isolated two DNA sequences, here named Zv516 and Zv817 (of 516 and 817 bp), in the common European lizard *Zootoca vivipara*, characterizing their nucleotide content, copy number, chromosomal location and conservation in different amniote taxonomic groups.

Molecular and bioinformatic analyses showed that Zv516 and Zv817 contain truncated traits of two different repeated interspersed DNA sequences, SINE Squam1 and TC1 Mariner, belonging to class I and II Transposable Elements (TEs), respectively. Quantitative dot blot evidenced that both TEs represent about 0.03% of the genome of *Z. vivipara*, with about 3200-4500 copies, and comparable quantities were found in different squamate groups. *Fluorescent In Situ Hybridization* (FISH) located both sequences on all chromosome pairs, with probes from Zv516 preferentially distributed on centromeric and telomeric regions, while probes from Zv817 were also on interstitial chromosome regions.

A different trait of Zv817 (of about 300 bp) was identified as an amniote Ultra-Conserved Element (UCE) occurring in reptiles, birds and mammals (identities from 86% to 100%), while no significant identities were found in Whole-Genome Shotgun (WGS) archives of amphibians, fishes and various tested invertebrate groups. Upstream and downstream regions (of about 1000 bp) of the same UCE are also conserved in different reptile groups with high identities at family level (>96%).

Phylogenetic analyses performed both with the isolated UCE and its contigs provided tree topologies which were largely consistent with accepted evolutionary relationships at different taxonomic level.

Further discussion will cover aspects of the genomic evolution of the isolated TEs and UCE.

**COMMON WALL LIZARD (*Podarcis muralis*) VS URBANIZATION**

MARKO MIRČ

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

NATAŠA TOMAŠEVIĆ KOLAROV

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

SRĐAN STAMENKOVIĆ

Faculty of Biology, University of Belgrade, Belgrade, Serbia

TANJA VUKOV

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

Urbanization is predominantly linked with negative effects on wildlife. Indeed, numerous human-induced environmental changes (e.g. habitat fragmentation and destruction, pollution, etc.) have a devastating effect on indigenous wildlife. However, urbanization, through creating pockets of subnatural habitats, can have a positive effect on indigenous species. In our study, we wanted to examine how urbanization level (urban, suburban and natural habitats) affects *Podarcis muralis* populations across the Vojvodina region, Serbia. We tested (A) lizard's pileus: (1) size and shape, (2) fluctuating asymmetry (FA), (3) allometry, (4) modularity, and (5) phenodeviant frequencies and (B) meristic traits (supraciliar plates, sublabial plates, subdigital lamellae of 4<sup>th</sup> hind limb toes, femoral pores): (1) fluctuating asymmetry (FA). Pileus examination gave some peculiar results: unexpectedly high FA level was observed in the natural population. On the other hand, analysis of meristic traits showed high FA in urban population. Importantly, some meristic characters revealed different patterns of FA, suggesting that caution must be practiced when deciding what characters are more appropriate for FA analysis. In addition, we built an ecological niche model for the populations inhabiting the Vojvodina region (consisted predominantly of anthropogenic habitats) and compared it with the one obtained for central and southern Serbia, where lizards mainly occupy natural habitats. With this comparison, we wanted to see what the similarities between natural and anthropogenic habitats are, and which environmental factors limit/enable common wall lizard to successfully inhabit urbanized areas.

**PHYLOGENETIC SYSTEMATICS OF *Bufo bufo* SPECIES GROUP IN TURKEY**

NURHAYAT ÖZDEMİR

Recep Tayyip Erdogan University, Rize, Turkey

CANTEKİN DURSUN

Recep Tayyip Erdogan University, Rize, Turkey

SERKAN GÜL

Recep Tayyip Erdogan University, Rize, Turkey

BİLAL KUTRUP

Karadeniz Technical University, Trabzon, Turkey

NAZAN ÜZÜM

Aydın Adnan Menderes University, Aydın, Turkey

Mediterranean Basin is an ideal laboratory for phylogeographic and biogeographic studies in Anatolia. The collision of the different tectonic plates and climatic oscillations in Quarternary Period left signature on the phylogeographical histories of species inhabiting the Mediterranean Basin. In recent years, different molecular published studies have pointed different results for the species distributing in this region. When the literature is investigated, the systematic uncertainty of the species (*Bufo bufo* and *Bufo verrucosissimus*) belonging to *Bufo bufo* species group distributing in Anatolia is outstanding in the Mediterranean Region. For determining phylogenetic relations and revealing distribution of *Bufo bufo* species group, 193 (96 ♀♀, 97 ♂♂) individuals were sampled from 25 different localities in Turkey. A portion of 544 bp of the mitochondrial 16 rRNA gene and 786 bp of the mitochondrial *cyt-b* genes were amplified. Purification of PCR products and sequencing was performed by Macrogen, Inc. Bayesian and Maximum Likelihood analyses were performed with 16S rRNA and *cyt-b* genes by using MrBayes 3.2.6 and RaxML v8.0, respectively. According to phylogenetic analyses, the samples of *Bufo bufo* species group in Turkey placed in two different clades. Samples of Mediterranean and northeast region placed in the same branch with samples of *B. verrucosissimus* from Georgia and Russia, and the rest of samples in *B. bufo* clade.

**SYSTEMATICS REVISION OF THE *Mesalina olivieri* SPECIES COMPLEX  
(SQUAMATA: LACERTIDAE) FROM NORTH-WEST AFRICA WITH THE  
DESCRIPTION OF ONE NEW SPECIES**

CRISTIAN PIZZIGALLI

CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto. Vairão, Portugal.  
Departamento de Biologia da Faculdade de Ciências da Universidade do Porto. Porto, Portugal

PIERRE-ANDRÉ CROCHET

CEFE, CNRS, University of Montpellier, University Paul Valéry Montpellier 3, EPHE, IRD, Montpellier, France

PHILIPPE GENIEZ

EPHE, PSL Research University, CNRS, UM, SupAgro, IND, INRA, UMR 5175 CEFE, 34293 Montpellier, France

FERNANDO MARTÍNEZ-FREIRÍA

CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto. Vairão, Portugal.

GUILLERMO VELO-ANTÓN

CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto. Vairão, Portugal.

JOSÉ CARLOS BRITO

CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto. Vairão, Portugal.  
Departamento de Biologia da Faculdade de Ciências da Universidade do Porto. Porto, Portugal

*Mesalina* is a genus of small xeric lizards distributed from Mauritania throughout the Saharo-Sindian deserts to the Indo-Iranian plateau. Previous phylogenetic studies within the genus highlighted the presence of cryptic diversity and described new *Mesalina* species from the eastern lineages of the genus. In this study, we investigated the diversity within the *Mesalina olivieri* species complex in North-West Africa, especially focusing on the Atlantic Sahara region (from Morocco to Mauritania). The genus is currently represented by three recognised species in this area: *M. olivieri*, *M. pasteuri* and *M. simoni*. Using an integrative taxonomy approach based on morphological (both pholidotic, coloration and pattern) and molecular (1 mtDNA and 4 nuDNA markers) datasets, we provide robust molecular and morphological evidence for the existence of at least four species delegate to the *M. olivieri* complex within this area, including an undescribed species in Mauritania. This new *Mesalina* species is sympatric with *M. pasteuri* and *M. guttulata* in Mauritania but differ from them genetically, morphologically and in habitat selection. Species distribution modelling suggests that the species occurs in the Adrar Atar plateau and neighbouring regions. The relatively wide distribution suggests that its conservation status can be assessed as Least Concern.

**NARROW-MOUTHED FROGS OF THE SUBFAMILY ASTEROPHRYINAE  
(ANURA: MICROHYLIDAE): AN ADDITION TO INDOCHINESE  
HERPETOFAUNA**

NIKOLAY A. POYARKOV

Biological Faculty, Department of Vertebrate Zoology, Lomonosov Moscow State University, Moscow, Russia  
CHATMONGKON SUWANNAPOOM

Division of Fishery, School of Agriculture and Natural Resources, University of Phayao, Phayao, Thailand

Asterophryinae is the most speciose subfamily within Microhylidae, comprising over 330 species distributed mostly in northern Australia, New Guinea, and adjacent Australasian islands. While Microhylidae are supposed to have originated from Gondwanaland, “the Antarctic route scenario” was proposed for asterophryines, suggesting that their ancestor colonized Australia via Antarctic land bridge and subsequently dispersed to Australasia, but was unable to cross the Wallace line. Kurabayashi et al. (2011) demonstrated that the genus *Gastrophrynoides* from Borneo and Malay Peninsula belongs to Asterophryinae. Our recent surveys in Indochina resulted in discovery of two new genera and four new species of Asterophryinae: *Siamophryne* from Thailand (Suwannapoom et al., 2018) and *Vietnamophryne* from Vietnam and Thailand (Poyarkov et al., 2018). These discoveries are of biogeographic importance: together with *Gastrophrynoides* the two new genera form a clade sister to all remaining Asterophryinae, thus strongly supporting the “out of Indo-Eurasia” biogeographic scenario for asterophryines, suggesting that the basal split of the subfamily may have occurred on the Eurasian mainland from where it dispersed to Australasia. Eurasian asterophryines demonstrate varying ecological specializations: *Siamophryne* is the only known to date troglophilous frog, with very peculiar tadpole morphology not reported for other anurans, while *Vietnamophryne* and *Gastrophrynoides* have semi-fossorial lifestyle; arboreal forms were also discovered. Presence of larval stage represents a fundamental difference between the Indochinese and Australasian “core” Asterophryinae. Further survey efforts demonstrated that diversity of Asterophryinae in Indochina is still underestimated. This study was carried out with financial support from the Russian Science Foundation (RSF grant № 19-14-00050).

**THE PHYLOGENETIC RELATIONSHIPS OF THE *SALAMANDRIDAE*  
(AMPHIBIA: URODELA): A HISTORY OF DEEP AND SHALLOW  
RETICULATIONS**

LOIS RANCILHAC

Zoological Institute, Technische Universität Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany

HERVÉ PHILIPPE

Centre for Biodiversity Theory and Modelling, UMR CNRS 5321, Station of Theoretical and Experimental Ecology, 2 route  
du CNRS, 09200 Moulis, France

MIGUEL VENCES

Zoological Institute, Technische Universität Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany

The Salamandridae are a prominent Holarctic group of salamanders of considerable interest in studies of toxicity and aposematism, courtship behavior, and molecular evolution. Although the relationships between the 21 currently recognized salamandrid genera have been the subject of numerous molecular phylogenetic studies, some nodes have remained controversial. We generated new transcriptomic (RNAseq) data for 20 salamandrid samples, which together with already published transcriptome data, were used to obtain a comprehensive nuclear gene perspective on salamandrid evolution. After thorough filtering and decontamination, our final phylogenomic dataset included 5,455 gene alignments for 40 taxa representing 17 of the 21 accepted genera and a total length of 9,546,906 bp. “State of the art” phylogenomic analyses recovered a consistent, well supported topology that mostly agreed with previously published studies. However, the position of some taxa conflicted with phylogenies obtained from complete mitogenomes, while receiving strong support in both cases. Hybridization analyses, conducted by using both phylogenetic network inference and introgression tests, revealed extensive gene flow, particularly within the so-called “Modern Newts”, consistently with mtDNA/transcriptomic incongruences. Indeed, several hybridization events seems to link the ancestral lineages of the genera *Calotriton*, *Triturus*, *Neurergus*, *Ichthyosaura* and *Ommatotriton*, while a more recent reticulation links *Lissotriton* and *Triturus*. Similar patterns seems to affect the Asian genera *Cynops*, *Paramesotriton* and *Pachytriton*, although our sparse sampling makes them difficult to interpret. These results demonstrates that extensive gene flow occurs across Salamandrids lineages, and highlight the importance of taking them into account for further phylogenetic and evolutionary studies.

**EVOLUTIONARY HISTORY OF THE LEAF-TOED GECKO *Euleptes europaea* REVEALS RECENT BIOGEOGRAPHIC CROSS-ROAD BETWEEN SARDINIA AND CORSICA**

DANIELE SALVI

University of L'Aquila, Coppito, Italy

MICHEL DELAUGERRE

Conservatoire du Littoral, Bastia, France

SALVADOR CARRANZA

Institute of Evolutionary Biology – CSIC, Barcelona, Spain

D. JAMES HARRIS

Centro de Investigação em Biodiversidade e Recursos Genéticos - CIBIO, Vairão, Portugal

The Corsica-Sardinia archipelago provides a simplified model for studying the response of insular and coastal temperate biotas to the cyclic contraction-expansion of habitats associated to Pleistocene climate and sea-level oscillations. We investigated the phylogeography and historical demography of the Leaf-toed Gecko *Euleptes europaea* using both mitochondrial and nuclear genetic markers. Phylogenetic analyses uncover several divergent mtDNA lineages with a clear geographic association and with a partial sorting at nuclear loci. These lineages likely diversified during the Pleistocene and show an allopatric pattern, suggesting a scenario of long-term isolation in multiple glacial refugia. Bayesian phylogeographic reconstruction of the spatial diffusion process suggests that during the last glacial phase the northern Sardinia lineage expanded northward and colonized Corsica.

The evolutionary history of *E. europaea* and other co-distributed amphibians and reptiles indicates a major role of Pleistocene climatic oscillations and sea-level dynamics in structuring genetic diversity patterns in the Corso-Sardinian biota.



**A NEW APPROACH TO BUILD THE AMPHIBIAN TREE OF LIFE**

ANA SERRA SILVA

Life Sciences Department, The Natural History Museum, London, United Kingdom;  
School of Earth Sciences, University of Bristol, Bristol, United Kingdom

MARK WILKINSON

Life Sciences Department, The Natural History Museum, London, United Kingdom

Previous efforts to build the Amphibian Tree of Life have relied heavily on supermatrix approaches, where large amounts of data are concatenated into a single computational-expensive phylogenetic analysis. With the ever-increasing numbers of described species, this approach lags behind taxonomic knowledge. Among the over 8000 described amphibian species, only half have been included in the latest published Amphibian Tree of Life. Taking advantage of the inherent scalability of amphibian orders (Gymnophiona: 212 species; Caudata: ~730 species; Anura: ~7060 species), we aim to test the use of supertree, and hybrid supertree/supermatrix approaches to build the Tree of Life. Preliminary phylogenetic analyses of caecilians have shown that supertree analyses are considerably faster (2.5 vs. 10 days), but produce less resolved trees than concatenated analyses. To increase supertree resolution we are attempting to optimize the gene trees prior to the supertree analyses, and to test a hybrid tree building approach, where supermatrix, not gene tree, analyses are run on subsets of closely related taxa, and the resulting phylogenies summarized with a supertree method. Continuing work on gene tree optimization and analyses of salamander and frog data, should show the suitability of a modular tree building approach. Additionally, the use of a supertree or hybrid approach would make the integration of molecular and morphological data in phylogenetic analyses easier, and allow for more extensive taxon sampling. In short, we hope to show that supertree and/or hybrid approaches can have the same resolution as supermatrix analyses, and able to close the gap between amphibian taxonomy and systematics.

**SPOTTED TOAD-HEADED AGAMAS OF THE *Phrynocephalus guttatus* COMPLEX:  
IN SEARCH OF SPECIES BOUNDARIES**

EVGENIYA N. SOLOVYEVA

Zoological Museum of the Lomonosov Moscow State University, Moscow, Russia

EVGENIY A. DUNAYEV

Zoological Museum of the Lomonosov Moscow State University, Moscow, Russia

NIKOLAY A. POYARKOV

Biological Department of the Lomonosov Moscow State University, Moscow, Russia

The spotted toad-headed agamas *Phrynocephalus guttatus* (Reptilia, Agamidae) inhabit different types of deserts on the vast territory, spreading from Kalmykia in Russia to the westernmost parts of China. *Phrynocephalus guttatus* s.l. actually represents one of the youngest species complexes of *Phrynocephalus* genus and includes several species and subspecies. The majority of them live in Eastern Kazakhstan, several inhabit Transbalkhash region: *P. kuschakewitschi* and *P. incertus*; areas of *P. alpherakii* and *P. melanurus* are nearby. The aim of our study was to specify the borders of ranges of *P. kuschakewitschii* и *P. incertus*, whether they are distinct or these two forms flow smoothly from one into another and if there is a hybridization. We studied 149 samples of spotted toad-headed agamas and we used fragments of mtDNA genes (668 b.p. of COI for all samples, 291 b.p. of cytb and 705 b.p. of ND4 for selected groups). We reconstructed phylogenetic trees with MrBayes ver. 3.2. The three mtDNA markers gave us different picture. In the COI phylogenetic tree, two Transbalkhash populations separated (Taukum and Kabanbay) and they group with *P. versicolor*, so here we observe either pseudogen influence or traces of old hybridization event between *P. guttatus* s. l. and *P. versicolor*. According to cytb results these two populations are mixed with *P. kuschakewitschii*, while according to ND4 data Taukum and Kabanbay again stay separately. More sequences of mtDNA, research on nuDNA markers are needed to clarify the situation. The research was supported by the Russian Science Foundation RSF, grant No. 19-14-00050.

**COMPARATIVE LANDSCAPE GENETICS REVEALS THE EVOLUTION OF VIVIPARITY REDUCES GENETIC CONNECTIVITY IN FIRE SALAMANDERS**

ANDRÉ LOURENÇO

CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto. Instituto de Ciências Agrárias de Vairão. Rua Padre Armando Quintas 7. 4485-661 Vairão Portugal

JOÃO GONÇALVES

CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto. Instituto de Ciências Agrárias de Vairão. Rua Padre Armando Quintas 7. 4485-661 Vairão Portugal

FILIPE CARVALHO

Department of Zoology and Entomology, School of Biological and Environmental Sciences, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa

IAN J. WANG

Department of Environmental Science, Policy, and Management, University of California, 130 Mulford Hall #3114, Berkeley, CA 94705 USA

GUILLERMO VELO-ANTÓN

CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto. Instituto de Ciências Agrárias de Vairão. Rua Padre Armando Quintas 7. 4485-661 Vairão Portugal

Evolutionary changes in reproductive mode may affect co-evolving traits, such as dispersal, though this subject remains largely underexplored. The shift from aquatic oviparous/larviparous reproduction to terrestrial viviparous reproduction in some amphibians entails skipping the aquatic larval stage and, thus, greater independence from water. Accordingly, amphibians exhibiting terrestrial viviparous reproduction may potentially disperse across a wider variety of sub-optimal habitats and increase population connectivity in fragmented landscapes compared to aquatic-breeding species. We investigated this hypothesis in the fire salamander (*Salamandra salamandra*), which exhibits both aquatic- (larviparity) and terrestrial-breeding (viviparity) strategies. We genotyped 426 larviparous and 360 viviparous salamanders for 13 microsatellite loci and sequenced a mitochondrial marker for 133 larviparous and 119 viviparous individuals to compare population connectivity and landscape resistance to gene flow within a landscape genetics framework. Contrary to our predictions, viviparous populations exhibited greater differentiation and reduced genetic connectivity compared to larviparous ones. Landscape genetic analyses indicate viviparity is partially responsible for this pattern, as water courses comprised a significant barrier only in viviparous salamanders due to their fully terrestrial life cycles. Agricultural areas and, to a lesser extent, topography also decreased genetic connectivity in both larviparous and viviparous populations. This study is one of the very few to explicitly demonstrate the evolution of a derived reproductive mode affects patterns of genetic connectivity and, consequently, population dynamics. Our findings open avenues for future research to better understand the eco-evolutionary implications underlying the emergence of terrestrial reproduction in amphibians.

**LANDSCAPE GENOMICS OF THE CASPIAN WHIPSNAKE (*Dolichophis caspius*)  
ACROSS EASTERN EUROPE AND WESTERN ASIA**

SARITA MAHTANI-WILLIAMS

Research Institute of Wildlife Ecology, Vetmeduni Vienna, Vienna, Austria  
Cardiff School of Biosciences, Cardiff University, Cardiff, UK

Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall, UK

WILLIAM FULTON

Research Institute of Wildlife Ecology, Vetmeduni Vienna, Vienna, Austria  
Cardiff School of Biosciences, Cardiff University, Cardiff, UK

AMELIE DESVARS-LARRIVE

Research Institute of Wildlife Ecology, Vetmeduni Vienna, Vienna, Austria

SARA LADO

Research Institute of Wildlife Ecology, Vetmeduni Vienna, Vienna, Austria

JEAN ELBERS

Research Institute of Wildlife Ecology, Vetmeduni Vienna, Vienna, Austria

BÁLINT HALPERN

MME Birdlife Hungary, Budapest, Hungary

GERGELY BABOCSAY

Mátra Museum of the Hungarian Natural History Museum, Gyöngyös, Hungary

MME Birdlife Hungary, Budapest, Hungary

ZOLTÁN TAMÁS NAGY

Independent Researcher, Berlin, Germany

PABLO OROZCO-TERWENGEL

Cardiff School of Biosciences, Cardiff University, Cardiff, UK

DÁVID HERCZEG

Lendület Evolutionary Ecology Research Group, Plant Protection Institute, Centre for Agricultural Research, Hungarian  
Academy of Sciences, Budapest, Hungary

JUDIT VÖRÖS

Department of Zoology, Hungarian Natural History Museum, Budapest, Hungary

PAMELA BURGER

Research Institute of Wildlife Ecology, Vetmeduni Vienna, Vienna, Austria

The Caspian whipsnake, *Dolichophis caspius* (Gmelin, 1789), inhabits a wide variety of habitats from Eastern Europe to Western Asia. Until now, genetic studies on this species have been scarce. We used double-digest restriction associated DNA (*ddRAD*) sequencing of 53 samples providing 17K single nucleotide polymorphisms (SNPs) to estimate genetic diversity, describe the effective migration patterns of different populations sampled from across the range of distribution, and detect genotypes and genes under selection by climatic factors. PCA, AMOVA, NeighborNet network and Admixture analysis identified 5-9 clusters among the studied populations. Estimated Effective Migration Surfaces revealed higher-than-average gene flow in most of the Balkan Peninsula, and lower-than-average gene flow along the middle section of the Danube River, where populations are located in small isolated patches. Landscape genomic analysis with *SamBada* identified a total of 751 selected genotypes that correlated with seven climatic variables (wind speed in April, annual mean temperature, isothermality, temperature annual range, mean temperature of the wettest quarter, annual precipitation and precipitation in the driest quarter) included in the model. Isothermality correlated with the highest number of selected genotypes (478) found in 41 genes, followed by annual range (127) and annual mean (87) of temperature. Annual precipitation had the least correlation with only two genotypes in noncoding regions. We conclude that environmental variables, especially the day-to-night temperature oscillation in comparison to the summer-to-winter oscillation, play a role in the distribution of Caspian Whipsnakes across their habitats.

**MICROSATELLITE GENOTYPES OF SEVEN HYBRIDOGNETIC UNISEXUAL SPECIES OF ROCK LIZARD AND THEIR PUTATIVE BISEXUAL PARENTS (GENUS *Darevskia* sp.) SUGGEST COMPLEX PATTERNS OF HYBRID ANCESTRY**

DAVID TARKHNISHVILI

Ilia State University, Tbilisi, Georgia

ALEXEY YANCHUKOV

Zonguldak Bülent Ecevit University, Zonguldak, Turkey

MARINE MURTSKHVALADZE

Ilia State University, Tbilisi, Georgia

SOFIKO KURDADZE

Ilia State University, Tbilisi, Georgia

NATIA BARATELI

Ilia State University, Tbilisi, Georgia

MARIAM GABELAIA

Ilia State University, Tbilisi, Georgia

MEHMET KÜRŞAT ŞAHİN

Hacettepe University, Faculty of Science, Department of Biology, Ankara, Turkey

Karamanoğlu Mehmetbey Üniversitesi, Faculty of Science, Department of Biology, Karaman, Turkey

KAMİL CANDAN

Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir, Turkey

YUSUF KUMLUTAŞ

Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir, Turkey

ÇETİN ILGAZ

Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir, Turkey

FARUK ÇOLAK

Zonguldak Bülent Ecevit University, Zonguldak, Turkey

FERHAT MATUR

Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir, Turkey

MERİÇ ERDOLU

Middle East Technical University, Faculty of Science, Department of Biology, Ankara, Turkey

MARİNE ARAKELYAN

Yerevan State University, Yerevan, Armenia

EDUARD GALOYAN

Moscow State University, Moscow, Russia

Our current best molecular genetic evidence providing insight into the hybrid origin of several distinct parthenogenetic species of *Darevskia* is based on allozymes and partial mtDNA sequences. We have genotyped all the seven known parthenogenetic forms: *D. armeniaca*, *D. dahli*, *D. unisexualis*, *D. uzzelli*, *D. bendimahiensis*, *D. sapphirina*, *D. rostombekovi* and their putative parental bisexual species (*D. mixta*, *D. raddei*, *D. portschinski*, *D. valentini*, *D. rudis*), each species represented by several geographic populations from Georgia, Eastern Turkey and Armenia, for 10 highly polymorphic microsatellite loci. Initially, we found higher similarity of alleles between the unisexual species and their respective putative maternal bisexual progenitors (*D. mixta* and *D. raddei*), with geographically proximal populations sharing the highest proportions of common alleles. A less clear pattern was observed on the paternal side, possibly caused by incomplete lineage sorting among the putative paternal species (*D. portschinski* and *D. valentini*). Bayesian clustering revealed three ancient ancestry components prior to the origin of parthenogenesis: maternal (i) *D. mixta*, (ii) *D. raddei* and paternal (iii) *D. valentini* + *D. portschinskii* + *D. rudis*. These clusters can then be detected in all parthenogens: however, only in a few cases the maternal and paternal ancestry components are represented in equal proportions. Instead, some unisexual species have 1:3 or 1:4 ancestry ratios, in favor of either maternal or paternal side. Remarkably, we found high level of coincidence of multilocus genotypes among the parthenogenetic species, even between distantly related forms. Various evolutionary scenarios that could have caused these complex patterns are discussed. This study was supported by a joint TÜBİTAK-SRNSF grant 216Z189.

**MULTILOCUS PHYLOGENY OF SMALL VIPERS (SUBGENUS: *Pelias*; GENUS: *Vipera*) BASED ON GENOME-GUIDED ddRAD NUCLEAR DNA MARKERS**

OLEKSANDR ZINENKO

V. N. Karazin Kharkiv National University, Kharkiv, Ukraine

GLIB MAZEPA

University of Lausanne, Lausanne, Switzerland

LISLE GIBBS

Ohio State University, Columbus, OH, USA

ULRICH JOGER

State Natural History Museum, Braunschweig, Germany

Small vipers assigned to the subgenus *Pelias* of genus *Vipera* constitute a difficult group for phylogenetic reconstruction due to high levels of range-wide variation within species and hybridization between recognized species. This is a young ( $\leq 7$  Mya) monophyletic group of taxa with a broad range across the temperate regions of Europe and Northern Asia. Many taxa in the group are highly variable with local populations having unique combinations of morphological characters that lead to their description as separate species. Recent work using ddRADseq loci has demonstrated the occurrence of distinct populations with mixed ancestry suggesting that hybridization may be an important mode of speciation in this group. This calls into question previous phylogenetic reconstructions for this group based on mtDNA because such markers do not account for hybridization when reconstructing relationships between lineages. Here, we describe the use of ddRAD-derived loci to reconstruct a phylogeny of species in the group in which homologous loci across species have been identified using the reference genome for *Vipera berus*. The phylogeny that we generate for these snakes has a topology which is in conflict with previous mitochondrial trees and represents the first robust hypothesis about origin and diversification of this group that is based on a multilocus nuclear DNA data set.



## Session: Methodological advances



**UNCOVERING THE GENOMIC AND METAGENOMIC POTENTIAL IN OLD  
ETHANOL-PRESERVED SNAKES**

MORTEN E. ALLENTOFT

Department of Biology, University of Copenhagen, Copenhagen, Denmark

CLAUS G. ZACHO

Department of Biology, University of Copenhagen, Copenhagen, Denmark

MARTINA A. BAGER

Department of Biology, University of Copenhagen, Copenhagen, Denmark

ASHOT MARGARYAN

Department of Biology, University of Copenhagen, Copenhagen, Denmark

PETER G. NIELSEN

Department of Biology, University of Copenhagen, Copenhagen, Denmark

ARNE R. RASMUSSEN

Affiliation, City, Nation

When using museum specimens for genome-scale analyses, favorable preservation conditions, combined with selective sampling and optimized laboratory protocols, are of crucial importance. Fish, reptiles, amphibians, and many invertebrate collections have often been preserved in ethanol for decades or centuries, and our knowledge on the genomic and metagenomic research potential of such material is limited. Here, we use ancient DNA protocols, combined with shot-gun sequencing to test the molecular preservation in liver, skin and bone tissue from five old (1842 to 1964) museum specimens of the Common garter snake (*Thamnophis sirtalis*). We assess the DNA quality by bioinformatically mapping the reads to a *T. sirtalis* reference genome. The DNA molecules are highly damaged with short average sequence lengths (38-64 bp) and high C-T deamination damage. Despite this, the samples display a relatively high endogenous DNA contents, ranging from 26% to 56%, revealing that genome-scale analyses are indeed possible from all specimens and tissues included here. Of the three tested types of tissue, bone show highest DNA quality, expressed as higher levels of endogenous DNA, longer DNA fragments, and lower contamination levels. Lastly, we demonstrate that these specimens display a diverse and tissue-specific microbial genetic profile, thus offering authentic metagenomic data despite being submerged in ethanol for many years. Our results emphasize that museum collections continue to offer an invaluable source of information - also in the era of genomics.

## **THE ROLE OF ARTIFICIAL SHELTERS IN THE STUDY OF ALPINE REPTILES IN THE STELVIO NATIONAL PARK**

**ANDREA BARBI**

Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, Via Accademia Albertina 13, I-10123 Torino, Italy

**DANIELE DELLE MONACHE**

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Via Ferrata 9, I-27100 Pavia, Italy

**OSCAR DONELLI**

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Via Ferrata 9, I-27100 Pavia, Italy

**MAURO FASOLA**

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Via Ferrata 9, I-27100 Pavia, Italy

**ROBERTA ROSSI**

Via Fatebenefratelli 4, I-10137 Torino, Italy

**ROBERTO SINDACO**

I.P.L.A. - Istituto per le Piante da Legno e l'Ambiente, Corso Casale 476, I-10132 Torino, Italy

**LUCA PEDROTTI**

ERSAF – Direzione Parco Nazionale dello Stelvio, Via De Simoni 42, I-23032 Bormio (SO), Italy

**DANIELE PELLITTERI-ROSA**

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Via Ferrata 9, I-27100 Pavia, Italy

Alpine habitat, due to its morphological and geographical configuration, makes extremely difficult to contact and monitor animal species. This is even more evident in the case of reptile species, whose monitoring schemes often failed due to the low encounter rate of target species. The employ of artificial shelters can be a suitable solution to overcome these problems. By their usage, we collected data of three target species (*Anguis veronensis*, *Zootoca vivipara*, *Vipera berus*) in the Stelvio National Park's Lombard area during a period of 5 years, from 2014 to 2018. Our aim was to assess whether population and distribution ranges of the species had changed during the monitoring period in relation to environment and climate variables. In order to describe population dynamics of the target species, we parameterized several mixture models on presence-absence data according to the Royle & Nichols formulation (2003) and included an exponential growth term to model the temporal variations across sites with respect to both elevation and annual temperature differences. In a similar way, temporal exponential growth models were applied to point counts via several N-mixture models. Both formulations included a linear and quadratic effect of elevation on year-one abundance, and a linear and quadratic effect of the sampling period on detectability. By comparing the results of both approaches, we finally propose preliminary data on the evolution of the reptile's community in the Stelvio National Park territory, underlining the effectiveness of artificial shelters as a suitable and reliable method to investigate its distribution.

**COUNT DATA FOR MONITORING SINGLE POPULATIONS: A  
HERPETOLOGICAL CASE STUDY AND GENERAL VALIDATION**

MARCO BASILE

Chair of Wildlife Ecology and Management University of Freiburg, Freiburg, Germany

SEBASTIANO SALVIDIO

Department of Earth Environment and Life Sciences University of Genova, Genova, Italy

JOHANNES PENNER

Chair of Wildlife Ecology and Management University of Freiburg, Freiburg, Germany

ANDREA COSTA

Department of Earth Environment and Life Sciences University of Genova, Genova, Italy

Population size is a fundamental state variable in ecology and wildlife monitoring. Classical techniques for abundance estimation, such as Capture-Mark-Recapture, are time and effort consuming. In the last decade, the analysis of repeated count data with N-mixture models gave great advantage for population abundance estimation. N-mixture models require repeated surveys at multiple locations, while often only counts from a single population in subsequent years are available. We applied time-for-space substitution in N-mixture model framework to estimate population size and trend for a 20 years long study on a single population of the European leaf-toed gecko (*Euleptes europaea* Gené, 1839). We compared N-mixture model results with Capture-Mark-Recapture estimates, finding good agreement between methods. We also tested the reliability of time-for-space substitution in N-mixture models against > 140000 simulated scenarios with varying combinations of abundance, study duration, survey effort, population dynamic and detection probability. We found that the application of time-for-space substitution in N-mixture models is a cost-effective and reliable method for long-term population monitoring, particularly in the case of isolated populations, when a classical meta-population design isn't feasible or when Capture-Mark-Recapture techniques can't be applied or afforded.

## HOW TO ANALYSE COLORATION IN AMPHIBIANS AND REPTILES?

SIMONE GIACHELLO

Dipartimento di Scienze e Politiche ambientali, Università degli Studi di Milano, Milano, Italy

ENRICO LUNGI

Institute of Zoology, Chinese Academy of Sciences, Beijing, China

Museo di Storia Naturale dell'Università degli Studi di Firenze, Sezione di Zoologia "La Specola", Firenze, Italy  
Natural Oasis, Prato, Italy

Animals' coloration is an important life history trait often representing the result of specific environmental pressures; therefore, it may represent an important source of information on species biology. We here describe a methodology useful to study the coloration in amphibians and reptiles using the Italian cave salamander (*Hydromantes italicus*) as model species. We focused on the dorsal coloration for two main reasons: first, the dorsum is the most visible part of the body (the ventral side is usually in proximity of the ground) and thus, it likely holds several ecological meanings; secondly, individuals can easily be still in their natural position. We analysed the dorsal coloration of 141 individuals from four different caves. In each cave, a photographic set was built to take standardized pictures of animals in their natural position; after each photographic session instruments were disinfected to avoid pathogen spread. Pictures were first calibrated using Pantone colour card, cleaned from flash lights and other impurities, and then broken up into three main colour clusters, obtaining the proportion (%) of pixels for each. The dataset can be analysed in several ways, for example using the analysis of variance to assess a potential variability between studied groups and/or with mixed models to infer on the possible ecological causes for particular colorations. In our specific case, the proportion of each cluster in *H. italicus* varied among studied populations, according to the life stage of individuals and to the elevation of studied caves.

**FUNCTIONAL MAGNETIC RESONANCE IMAGING AS NOVEL TOOL TO  
STUDY ANURAN COGNITION?**

EVA RINGLER

University of Veterinary Medicine, Vienna, Vienna, Austria

MELISSA COATES

University of California, Los Angeles, Los Angeles, USA

ARIADNA COBO-CUAN

University of California, Los Angeles, Los Angeles, USA

NEIL G. HARRIS

University of California, Los Angeles, Los Angeles, USA

PETER M. NARINS

University of California, Los Angeles, Los Angeles, USA

Anuran amphibians are common model organisms in bioacoustics and neurobiology. To date, however, most available methods for studying auditory processing in frogs are highly invasive and thus do not allow for longitudinal study designs, nor do they provide a global view of the brain, which substantially limits the questions that can be addressed. We recently used in vivo manganese-enhanced MRI (MEMRI) to identify areas in the frog brain that are responsible for auditory processing. Our results show that stimulation with band-limited noise did not result in the same activation patterns than species-specific calls, indicating that signals with contrasting social relevance are differentially processed in these areas of the amphibian brain. We suggest MEMRI as a powerful novel approach to studying brain activity with high spatial resolution in frogs.

**EVALUATION OF A METHOD TO MONITOR A POPULATION OF THE MOROCCAN PAINTED FROG (*Discoglossus scovazzi*) UNDER EXTREME ENVIRONMENTAL CONDITIONS (HIGH ATLAS, MOROCCO).**

MOHAMED AMINE SAMLALI

Biodiversity and Ecosystem Dynamics Laboratory. University of Marrakech Cadi Ayyad, Faculty of Science Semlalia, Marrakech, Morocco.

ABDERRAHIM S'KHIFA

Biodiversity and Ecosystem Dynamics Laboratory. University of Marrakech Cadi Ayyad, Faculty of Science Semlalia, Marrakech, Morocco.

TAHAR SLIMANI

Biodiversity and Ecosystem Dynamics Laboratory. University of Marrakech Cadi Ayyad, Faculty of Science Semlalia, Marrakech, Morocco.

Population monitoring is a necessary prerequisite for assessing population trends of species. They are also essential for a proper assessment of conservation actions for threatened species. In this regard, monitoring methods should be developed that reflect population fluctuations over time. From this point of view, amphibians pose particular difficulties due to their nightlife, which explains the limited monitoring currently in place for this group. The ongoing study in the High Atlas in Oukaimeden (2600 m) aims to test a monitoring method applicable to a discrete and threatened species, the Moroccan painted frog – *Discoglossus scovazzi*. The method is based on the conduct of capture-mark-recapture (CMR) operations, and animal tagging consists of subcutaneous injection of electronic microchips (Nonatec Tags: 5 mm in length, weighing 0.1 g) with a unique bar code. The results already obtained show that the technique is well suited to the objectives pursued. It is easy to set up, and no risk is incurred by the animals. The large majority of tagged individuals were found as indicated by the first three years of follow-up (2016-2018). The method should make it possible to understand the ecological requirements of the species, and thus the impact of anthropogenic disturbances on population dynamics, and to draw the implications in terms of conservation to be undertaken in degraded environments.

This research was funded by the Hassan II Academy of Sciences and Technology (ICGVSA project)

**USE OF A WIRELESS ULTRASOUND PROBE AS A PORTABLE, NON-INVASIVE METHOD FOR STUDYING REPRODUCTIVE BIOLOGY IN WILD POPULATIONS OF THE ASP VIPER (*Vipera aspis*)**

MARCO SASSOÈ-POGNETTO

Department of Neuroscience, University of Torino, Torino, Italy

SONIA ACIERNO

Department of Neuroscience, University of Torino, Torino, Italy

SILVESTRO ROATTA

Department of Neuroscience, University of Torino, Torino, Italy

Ultrasonography has been widely used to study reproduction in reptiles, providing accurate information on clutch size and the dynamics of embryonic development. However, despite the introduction of light portable equipment, ultrasonography has been used in the field on only a limited number of species, most being tortoises and lizards. Field studies are required to investigate patterns of reproduction over time in wild populations, as well as to evaluate geographic variation of reproduction dynamics within individual species.

In this study, we present preliminary data on the reproductive ecology of the asp viper (*Vipera aspis*) in northwestern Italy using the capture-recapture method and ultrasound imaging. Female vipers were captured during the summer and individually identified by head scale patterns and markings. They were scanned ventrally and the number of embryos was estimated, while ultrasound images and videos were recorded. Ultrasound imaging was performed with a portable, pocket-sized (234 g) wireless ultrasound probe (Color Doppler OTE Linear L 102 CD; 7.5-10 MHz) interfaced with a tablet with a dedicated app. Vipers were then released at the exact capture site after collecting data on body size and weight.

We validate wireless ultrasound scanners as a non-invasive, effective tool for ultrasonic investigations in the field. Wireless probes are light and compact, which facilitates carriage in rugged terrain. Moreover, the absence of cables simplifies the maneuvers to be made on a small, and even potentially dangerous snake. Importantly, ultrasound scans can be performed at the capture site, thus minimizing restraint time and handling of gravid females.







## Session: Morphology and physiology

**EVOLVING TRENDS IN SNAKE VENOM RESEARCH: A REVIEW OF THE LAST 60 YEARS OF PUBLICATIONS**

IGNAZIO AVELLA

Faculty of Sciences of the University of Porto, Porto, Portugal

WOLFGANG WÜSTER

Molecular Ecology and Fisheries Genetics Laboratory, School of Biological Sciences, Bangor University, Bangor, United Kingdom

JUAN J. CALVETE

Evolutionary and Translational Venomics Laboratory, Institute of Biomedicine of Valencia, Valencia, Spain

FERNANDO MARTÍNEZ-FREIRÍA

CIBIO - Research Center in Biodiversity and Genetic Resources of the University of Porto, Vairão, Portugal

Snakebite is a globally neglected disease, only recently recognized by the World Health Organization. Despite the still low attention paid by health agencies and pharmaceutical companies to snakebite-related issues, venom research has grown consistently in recent years, being revolutionized by the introduction of new analytical tools (e.g. genomics, transcriptomics and proteomics). In this work, we aim to identify the trends and changes of snake venom research over time, and relate findings to the diversity of medically important venomous snakes. We reviewed 184 articles published between 1960 and 2019, related to this topic. We defined macro-categories describing both the topics and the analytical approaches reported in each article, identified the most studied taxa and detected the countries where snake venom research was developed. By cross-checking different databases, we looked at which dangerousness category the about 200 medically important venomous snake species are assigned to and which and how many effective antivenoms are reported for each. Snake venom research sharply increased in the early 2000s, with articles focusing on venom characterization and comparative venomics. Most of them focused on American species, with Crotalinae being the most studied group. The analysed databases reported fragmented information about most of the medically important snakes and available antivenoms. Despite the increasing trends in snake venom research, public databases need to be updated and future studies should increasingly focus on antivenom development and analysis of venom from more diverse snake taxa, particularly in countries where snakebite impact is the most severe.

**REPEATABILITY BUT NOT HERITABILITY OF METABOLIC RATE IN THE  
ALPINE NEWT**

SENKA BAŠKIERA

Department of Botany and Zoology, Masaryk University, Brno, Czech Republic

LUMÍR GVOŽDÍK

Institute of Vertebrate Biology of the Academy of Sciences of the Czech Republic, Brno, Czech Republic

Standard metabolic rate (SMR) represents the minimum amount of energy required to maintain life functions of an ectotherm at a given body temperature. It belongs among basic life history traits affecting the allocation of acquired energy into growth, survival, and reproduction. Its adaptive evolution assumes lifetime repeatability and heritability. Studies examining both assumptions are rare and virtually missing in amphibian physiology. We examined seasonal and long-term repeatability, as well as two heritability estimates, using full-sib comparison and parent-offspring regression, of SMR in the alpine newt, *Ichthyosaura alpestris*. Using intermittent respirometry, we repeatedly measured SMR in both newt cohort for five years since metamorphosis, and its metamorphosed offspring. SMR was consistently repeatable across one season and the whole measurement period. Sibling juveniles had more similar SMR than unrelated offspring indicating broad-sense heritability in this trait. Offspring and mid-parent values showed no similarity, and accordingly no narrow sense heritability of SMR. This suggests that the sibling similarity and long-term repeatability of this trait is caused by phenotypic plasticity and (or) dominance genetic variation rather than by additive genetic variation. We conclude that SMR fulfills assumption for phenotypic selection but the evolutionary potential of SMR is rather limited in this species. Our results have important implications for understanding sources of individual variation and eco-evolutionary significance of SMR in newts and other slow-living ectotherms.

**CHRONOLOGY OF LIMB REGENERATION IN CAPTIVE BORN JUVENILE OF THE EUROPEAN BLIND CAVE SALAMANDER**

LILIJANA BIZJAK MALI

Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia

KATJA DOLENC BATAGELJ

Postojnska Jama PLC

PRIMOŽ GNEZDA

Postojnska Jama PLC

KATARINA KANDUČ

Postojnska Jama PLC

STANLEY K. SESSIONS

Department of Biology, Hartwick College, Oneonta, NY, USA

[lila.bizjak@bf.uni-lj.si](mailto:lila.bizjak@bf.uni-lj.si)

A recent egg-laying event by captive *Proteus anguinus* at the Postojna Cave provided the opportunity to implement “optimal artificial” (OA) conditions for raising this vulnerable amphibian species in captivity. After three years under OA conditions, 92% of the juveniles continue to thrive and are now almost 11 cm in total length. These captive-raised juveniles allow us to observe the dynamics of their growth and behavior. At 1.5 years the juveniles began to exhibit aggressive behavior, and one individual had part of its hind limb chewed off, which allowed us to observe the process of limb regeneration. Five days following the injury, the damaged portion of the limb detached followed by wound healing that was completed within 7 days. The limb stump was initially swollen and inflamed. The inflammation disappeared after 1.3 months and the swelling was gone by 7.7 months. A blastema formed after 6 weeks and full regeneration took almost 11 months. Overall, limb regeneration in *Proteus* is similar to other salamanders but occurs at a much slower rate. In adult *Proteus* it took more than 1 year after wound healing for the limb to begin to regenerate. Occasionally, regeneration resulted in extra or missing digits or other deformities. This slow rate of regeneration in *Proteus* is probably due to its large genome size (approx. 47.9 Gb of DNA) which, in combination with lower temperature, slows cellular growth rate. As far as we know, this is the first time that limb regeneration has been described chronologically in a *Proteus* juvenile.

## HIGH OR LOW? A TEST OF THE RELATIONS BETWEEN ALTITUDE AND KERATINIZED SPINES

CANTEKİN DURSUN

Recep Tayyip Erdogan University, Rize, Turkey

NURHAYAT ÖZDEMİR

Recep Tayyip Erdogan University, Rize, Turkey

SERKAN GÜL

Recep Tayyip Erdogan University, Rize, Turkey

BİLAL KUTRUP

Karadeniz Technical University, Trabzon, Turkey

NAZAN ÜZÜM

Aydın Adnan Menderes University, Aydın, Turkey

Bufonidae is a family with stereotypical characters as dry warty skin which may bear keratinized spines. Many researchers related the intensity of spine presence to climatic conditions as local adaptation to dry environment or sexual differences. However, these studies have not examined the relation between altitude and the level of keratinized spines.

In this study, we aimed at testing the relations between these parameters. In this context, 74 adult female *B. bufo* individuals were sampled from 19 different localities in Turkey. To determine and categorize the intensity of presence of keratinized spines, photos were taken of the lateral side of the head. Specimens were categorized by following the relevant literature (Arntzen et al., 2013). The altitude of each sampling locality was also recorded. To categorize altitudes, the distribution range obtained from fieldwork data was divided into four subjective categories by taking each 500 meter as a reference step. All categories were coded by numbers and the Fisher's exact Test was used to test relations between altitude and keratinized spines. A statistically significant correlation was found between altitude and the intensity of occurrence of keratinized spines ( $P < 0.05$ ). 87.5% of specimens which have heavily keratinized spines was observed in the low altitudes. Contrary to what is reported in the literature, specimens with heavily keratinized spines were located at the north of Turkey, an area with temperate climatic conditions and sufficient precipitation. This study was supported by TÜBİTAK under project number 114Z823.

**QUANTITATIVE AND STRUCTURAL VARIATION IN SKIN SECRETED TOXINS  
ACROSS POPULATIONS OF THE FROG BOMBINA PACHYPUS (ANURA:  
BOMBINATORIDAE)**

LUCA COPPARI

S.H.I. Sezione interregionale Umbria – Marche, Perugia, Italy

SUNITA JANSSENSWILLEN

Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel, Brussels, Belgium

TIJN RAAIJMAKERS

Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel, Brussels, Belgium

KIM ROELANTS

Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel, Brussels, Belgium

Skin secretions of amphibians have been intensively studied because of their pharmacological properties and their potential use in clinical applications. In many species, the substance secreted contains different peptide families, which are stored in skin granular glands and released when the animal is stressed, for example when under attack by a predator. Although the skin secretion of many species has been molecularly characterised, any insight in natural variation within species and/or populations is absent. Understanding what influences natural secretion variation could improve the knowledge in evolutionary processes and forces that drive their adaptive evolution. Here, skin secretion variation is examined in eight populations of *Bombina pachypus*, broadly covering the species' distribution range. Variation in poison production, as well as in the concentration of the key toxin bradykinin, are examined in light of intrinsic (morphological) and extrinsic (environmental) variables. Latitude, altitude and sexes turn out to be variables that mostly influence poison production, with an increase in poison quantity towards the south and towards higher altitudes. Potential explanations for higher poison production in males are further discussed.

**EVOLUTION AND CAUSES OF VARIABILITY IN EVAPORATIVE WATER LOSS IN EYELID GECKOS (EUBLEPHARIDAE)**

MARTIN HLUBEŇ

Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

LUKÁŠ KRATOCHVÍL

Department of Ecology, Faculty of Science, Charles University, Prague, Czech Republic

LUMÍR GVOŽDÍK

Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic

ZUZANA STAROSTOVÁ

Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

Maintaining appropriate water balance is crucial for terrestrial animals and it is closely connected with the ability to colonize various habitats. In squamate reptiles, the major proportion of water loss can be attributed to cutaneous and respiratory water loss. Accordingly, total evaporative water loss (TEWL) might be influenced by body size, standard metabolic rate (SMR) and morphology of scales covering the body. We examined the association between these traits, and cutaneous water loss (CWL) and TEWL in the eyelid geckos (Eublepharidae), i.e. the group exhibiting large variation in body size and habitat use. According to the ancestral state reconstruction, the eublepharid ancestor had medium level of TEWL and the evolutionary shifts from this ancestral state involved both increase and decrease of TEWL. Body mass, CWL and TEWL were associated both within and among species. Departures from the common relationship between TEWL and body mass among species was strongly correlated with habitat aridity; however, they were not correlated with variability in SMR and scale morphology. Therefore, it seems that adaptive shifts in TEWL were not associated with changes in metabolic rate and scalation, which are the most frequently considered candidate mechanisms for adaptive modifications of this trait in reptiles. We propose other potential factors driving TEWL evolution in geckos.

**PATTERNS OF CORRELATIONS AND LOCOMOTOR SPECIALIZATION IN ANURAN LIMBS: ASSOCIATION WITH PHYLOGENY AND ECOLOGY**

TAMARA PETROVIĆ

Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia

TANJA VUKOV

Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia

NATAŠA TOMAŠEVIĆ KOLAROV

Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia

The anuran saltatory locomotion has specific functional requirements achieved through certain intra- and inter-limb proportions. Here we analyzed pattern and degree of morphological integration in limbs of ten anuran species in order to reveal the relationship of shared developmental programs of serially homologous structures and locomotor specialization. Our main objectives were to examine whether developmental and functional differences in forelimb and hindlimb were associated with reduced covariation between limbs, and to reveal patterns of correlation at the species level considering the roles played by the evolutionary history (phylogeny) and ecology (lifestyle and habitat use). Results showed shared patterns of functionally based morphological integration, with increased correlations in elements within limbs and reduced correlations between limbs. This was mainly based on strong correlations between proximal elements, humerus-radius-ulna and femur-tibiofibula. The changes of correlation patterns are shown to be dissociated from phylogeny. On the other hand, they are to some extent shaped by habitat use and locomotion, as the species with similar locomotor behaviour also tend to have stronger similarity in integration patterns. The results from this study provide an insight into the processes underlying the evolutionary change of anurans limbs, highlighting the function as main factor that shaped morphological integration of the examined species.



**RANID HERPESVIRUS 3 AND BUFONID HERPESVIRUS 1, TWO NEW VIRAL AGENTS ASSOCIATED WITH PROLIFERATIVE SKIN DISEASE IN FREE RANGING COMMON FROGS (*Rana temporaria*) AND COMMON TOADS (*Bufo bufo*) IN EUROPE**

FRANCESCO C. ORIGGI

Fiwi-University of Bern, Bern, Switzerland

BENEDIKT SCHMIDT

University of Zurich-Karch, Zurich-Neuchatel, Switzerland

PETRA LOHMANN

Veterinarian, Forch, Switzerland

THOMAS WAHLI

Fiwi-University of Bern, Bern, Switzerland

MICHAEL STOFFEL

Division of Veterinary Anatomy, University of Bern, Bern, Switzerland

We have recently discovered two new viral agents named *Ranid herpesvirus 3* and *Bufo herpesvirus 1* associated with proliferative skin disease in free-ranging common frogs (*Rana temporaria*) and toads (*Bufo bufo*) in Europe. The two diseases show striking similarities including the affected tissue, the nature of lesion, the association with a herpesvirus and the seasonality. On the other side, specific differences include the absence of keratinization in the affected frogs and its presence in the affected toads, the pigmentation of the affected skin in frogs (gray) and toads (brown) and the associated virus. The two viruses have a similar genomic architecture although *Bufo herpesvirus 1* is about 50 Kb shorter than *Ranid herpesvirus 3*. Both viruses contain putative immunomodulatory genes, which might play a role in the pathogenesis of the disease. However, it is not clear, if the infection with any of these viruses might predispose the host to infections by other pathogens or if it could worsen the clinical outcome of ongoing diseases. Both viruses and associated diseases have been discovered and characterized in Switzerland, however, previous investigations and anecdotal reports suggest that these diseases might have been circulating also in other European countries for at least 25 years. The significance of *Ranid herpesvirus 3* and *Bufo herpesvirus 1* in amphibian disease ecology is unknown, however, in times of global amphibian decline any microbial organism associated with disease such as *Ranid herpesvirus 3* and *Bufo herpesvirus 1* warrants a thorough assessment as possible stressor.

**POPULATION STRUCTURE AND VENTRAL POLYCHROMATISM OF *Helicops infrataeniatus* JAN, 1865 (SERPENTES, DIPSADIDAE) IN SUBTROPICAL BRAZIL**

RUTH ANASTASIA REGNET

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

FERNANDO MARQUES QUINTELA

Universidade Federal do Rio Grande, Rio Grande, Brazil

DANIEL LOEBMANN

Universidade Federal do Rio Grande, Rio Grande, Brazil

DENNIS RÖDDER

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

Assessments of the population structure of snakes are commonly difficult due to low detectability. Herein, we had the opportunity to determine the demographic structure, sex ratios and dimorphism and ventral polymorphism in a population of *Helicops infrataeniatus* based on 720 specimens, which were killed during a flood event in south Brazil. The population was composed by 51.1% adults, 47.6% juveniles and 1.3% newborns and the sex ratio was 45.4% females, 52.2% males, and 2.4% were of unidentifiable sex. Population was composed by 51.1% adults, 47.6% juveniles and 1.3% newborns. The total length (ToL) of females ranged from 18.4 to 78.2cm; Snout-vent length (SVL) ranged from 13.3 to 62.5cm; Tail length (TaL) ranged from 4.0 to 18.5cm; Sub-caudal scales ranged from 55 to 73; and weight ranged from 2.2 to 191g. ToL of males ranged from 19.7 to 67cm; SVL ranged from 13.9 to 46.7cm; TaL ranged from 4.9 to 21cm; sub-caudal scales ranged from 74 to 92; and weight ranged from 2.3 to 61.3g. Non-parametric t-tests detected highly significant differences between treatments tested (females vs. males), suggesting sexual dimorphism both in the number of sub-caudal scales and in the TaL:ToL ratios. Length-weight relationships analysis detected high determination coefficients for both sexes. There was neither a significant difference in the frequency of ventral staining patterns among sexes (38.4% checkered, 35.8% trilinear and 25.6% intermediate) nor in ventral coloration (reddish 62.2%, 26.2% yellowish and 11.6% with both tones). This is the first study providing detailed population level data on *H. infrataeniatus*.

**ENERGETIC EFFICIENCY OF METAMORPHOSIS IN *Rana temporaria***

KATHARINA RUTHSATZ

Institute for zoology, university of Hamburg, Hamburg, Germany

KATHRIN DAUSMANN

Institute for zoology, university of Hamburg, Hamburg, Germany

STEFFEN REINHARDT

Institute for zoology, university of Hamburg, Hamburg, Germany

TOM ROBINSON

Institute for zoology, university of Hamburg, Hamburg, Germany

NIKITA SABATINO

Department of life sciences, Hamburg university of applied sciences, Hamburg, Germany

MYRON PECK

Institute of hydrobiology and fisheries science, university of Hamburg, Hamburg, Germany

JULIAN GLOS

Institute for zoology, university of Hamburg, Hamburg, Germany

Environmental change exposes wildlife to an array of environmental stressors with the ability to disrupt endocrine function of the hypothalamus-pituitary-thyroid axis. In this study, we investigated how the alteration of thyroid hormone (TH) levels due to exposure to the environmentally relevant endocrine disruptor sodium perchlorate (SP; inhibitory) and exogenous L-thyroxin (T4; stimulatory) affects metabolic costs and energy allocation during and after metamorphosis in *R. temporaria*. We further tested for carry-over effects of endocrine disruption during larval stage on juvenile performance. Energy allocated to development was negatively related to metabolic rate and thus, tadpoles exposed to T4 could allocate 24 % less energy to development during metamorphic climax than control animals. Therefore, the energy available for metamorphosis was reduced in tadpoles with increased TH level by exposure to T4. We suggest that differences in metabolic rate caused by altered TH levels during metamorphic climax and energy allocation to maintenance costs might have contributed to a reduced energetic efficiency in tadpoles with high TH levels. Differences in size and energetics persisted beyond the metamorphic boundary and impacted on juvenile performance. *Energetic efficiency varied between treatments due to differences in size allocation of internal macronutrient stores.* Altered TH levels as caused by several environmental stressors lead to persisting effects on metamorphic traits and energetics and, thus, caused carry-over effects on performance of froglets. We demonstrate the mechanisms through which alterations in abiotic and biotic environmental factors can alter phenotypes at metamorphosis and reduce lifetime fitness in these and likely other amphibians.

**HOW (WILL) ECTOTHERMS COPE WITH CHANGING ENVIRONMENTS? A TEST WITH A LIZARD UNDER CONTRASTING ECOLOGICAL PRESSURES**

NINA SERÉN

CIBIO/InBIO Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal

RODRIGO MEGÍA-PALMA

CIBIO/InBIO Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal

FABIO M. GUARINO

University of Naples Federico II, Naples, Italy

ANAMARIJA ŽAGAR

National Institute of Biology, Ljubljana, Slovenia

TATJANA SIMČIČ

National Institute of Biology, Ljubljana, Slovenia

CATARINA PINHO

CIBIO/InBIO Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal

MIGUEL A. CARRETERO

CIBIO/InBIO Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal

Understanding how species respond to ever-changing environmental conditions is a major topic in evolutionary biology and can be the key for planning species conservation strategies. However, comprehensive studies integrating several sources of evidence (e.g. physiology, genomics) are still uncommon for non-model organisms in natural settings. One way to overcome this gap is to focus on populations across steep environmental gradients. These gradients are likely to promote phenotypic responses (either short-term plastic responses or long-term evolutionary shifts) to local conditions. Our model for this study is the endemic Canarian lizard from Tenerife (*Gallotia galloti*), which occurs from sea-level to an elevation of approximately 3700 meters. We sampled populations along this altitudinal interval and obtained spatial patterns of phenotypic variation on multiple traits (size, growth rate, age, telomere length, potential metabolic activity, corticosterone, parasites, thermal preferences and hydric loss). Our results indicate that lizards conserve the same daily patterns of preferred temperatures and water loss rate across altitudes suggesting plasticity of thermal and hydric ecology in the field. However, telomere length (a proxy for physiological stress) decreased with increasing altitude and potential metabolic activity was lower in the populations from 3500, 2200 and 100 meters above sea-level. This provides some evidence of physiological stress in extreme altitudes, but some between-population differences were not necessarily linked to elevation. Ongoing genomic analyses and translocation experiments will shed light on the drivers of these responses disentangling the roles of phenotypic plasticity and adaptation in coping with environmentally extreme settings in this lizard system.

**ECOPHYSIOLOGICAL TRAITS OF LACERTIDAE LIZARDS IN EXTREME ENVIRONMENTS IN MOROCCO**

ABDERRAHIM S'KHIFA

Biodiversity and Ecosystem Dynamics Laboratory. University of Marrakech Cadi Ayyad, Faculty of Science Semlalia, Marrakech, Morocco.

MOHAMED AMINE SAMLALI

Biodiversity and Ecosystem Dynamics Laboratory. University of Marrakech Cadi Ayyad, Faculty of Science Semlalia, Marrakech, Morocco.

TAHAR SLIMANI

Biodiversity and Ecosystem Dynamics Laboratory. University of Marrakech Cadi Ayyad, Faculty of Science Semlalia, Marrakech, Morocco.

Cold climates constitute a particular challenge for ectotherms. However, several squamate species have been able to colonize environments characterized by extreme environmental conditions (high altitudes). Studying thermophysiology of these animals is important to better understand the factors that influence their distribution and climatic sensitivity. Here we report on an ecophysiological study on three sympatric species, with different biogeographic affinities, living in the High Atlas at Oukaimeden. Measurements of body temperature in the field and in the laboratory were carried out in three Lacertidae species using a laser thermometer. The results obtained show that there are significant differences between the species studied, despite the many similarities (Phylogeny, morphology...). In addition, the preferred temperature in *Atlantolacerta andreanszkyi* is  $33.51 \pm 2.11$  °C, whereas it is  $34.84 \pm 0.84$  °C for *Scelarcis perspicillata* and  $34.39 \pm 1.30$  °C for *Podarcis vaucheri*. Thermal and hydric characterization of microhabitats was recorded during the study period every 30 minutes, using dataloggers installed in key areas (micro-habitats in full sun and in the shade). Evaporation water loss rates were estimated for the various lizards. Statistical analysis shows that there is no significant difference between the studied species, and that *A. andreanszkyi* has the highest evaporation water loss rate compared to the other species, with a loss of 7% of its initial weight, followed by *S. perspicillata* with a rate of 6% and *P. vaucheri* losing up to 4% of its weight initial. This key information can serve as a basis for understanding the mechanisms of responses of living beings to global changes. This research was funded by the Hassan II Academy of Sciences and Technology (ICGVSA project)

**COLOR IN FOCUS - CAN THE SAND LIZARD STILL SURPRISE US?**

RADOVAN SMOLINSKÝ

Department of Biology, Faculty of Education, Masaryk University, Brno, Czech Republic

TEREZA DRAČKOVÁ

Institute of Biostatistics and Analyses, Masaryk University, Brno, Czech Republic

ZUZANA HIADLOVSKÁ

Institute of Animal Physiology and Genetics, Czech Academy of Sciences, v.v.i., Brno, Czech Republic

MATEJ DOLINAY

Institute of Vertebrate Biology, Czech Academy of Sciences, v.v.i., Brno, Czech Republic

NATÁLIA MARTÍNKOVÁ

Institute of Vertebrate Biology, Czech Academy of Sciences, v.v.i., Brno, Czech Republic

Institute of Biostatistics and Analyses, Masaryk University, Brno, Czech Republic

Coloring of an organism facilitates communication of its fitness, social status within its group, as well as provides interspecific communication. Contrary to the role of coloring in intraspecific communication, the conspicuousness of the individual can influence the outcome of predator-prey interactions. Thus, the complex trade-off of an individual follows the main goal to genetically contribute to the next generation through attracting sexual partners and avoiding predators. The risk of being exposed to the predator increases with the predator's ability to perceive differences in a particular color spectrum and decreases with the ability of an individual to blend with the background of the environment. Natural selection should favor color aberrations that closely match the background in the environment. The sand lizard (*Lacerta agilis*) inhabits a wide range of biotopes within its geographical range, where it occurs in multiple color forms. Moreover, the coloration of sand lizards changes during their active season depending on sex and age. Previous studies have used photospectrometer data or other partial measurement methods to investigate sand lizard color polymorphism. We used quantitative methods of image processing to study totality of colors on sand lizards. To eliminate the effect of color pattern, we aggregated pixels of the respective colors. We compared seasonal color changes and differences of standardized color aberrations from the normotypic color type. Using this method, we created a mechanistic evolutionary model of sand lizard color.

**MORPHOLOGICAL INTEGRATION OF THE CRANIUM AND AXIAL SKELETON  
IN EUROPEAN NEWTS**

ALEKSANDAR UROŠEVIĆ

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

MAJA AJDUKOVIĆ

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

JAN W. ARNTZEN

Naturalis Biodiversity Center, Leiden, The Netherlands

ANA IVANOVIĆ

Institute of Zoology, Faculty of Biology, University of Belgrade, Belgrade, Serbia

Using micro-CT scanning and 3D geometric morphometrics of newt craniums and axial skeletons (first three vertebrae) we explored the pattern of morphological integration. We tested if i) directly connected serially homologous structures are more integrated than separated ones and ii) morphological integration coincides with regional differentiation. We applied a multilevel approach by analyzing patterns of integration at static and evolutionary levels, i.e., within and between species respectively. At the static level we choose the genus *Triturus* as a representative monophyletic group. We analysed between-individual variation in shape to detect functional modules and within-individuals the asymmetric component of variation in shape to detect developmental modules. At the evolutionary level, 17 species from five genera were analysed in phylogenetic context and taking effects of allometry on modularity and integration into account. We found that allometry is an important integrating factor in serially homologous structures. At the static level and after the correction for allometry, functional integration between the cranium and first vertebrae was weak but statistically significant between all elements, and developmental integration was significant between the cranium and the atlas and first and second trunk vertebrae. At the evolutionary level, the cranium, atlas and trunk vertebrae separate as three different modules. Our results suggest that, at the evolutionary level, morphological integration coincide with regional and functional differentiation of the axial skeleton. This allows the relatively independent evolution of the cranial skeleton and the vertebral column, separate of the significant functional and developmental integration at the static level.







# Workshop: Herpetofauna in agricultural landscapes

**SOME FIELDS ARE BETTER THAN OTHERS. ECOLOGICAL EFFECTS OF AGRICULTURAL MANAGEMENT ON LIZARDS**

MARTA BIAGGINI

Museo di Storia Naturale dell'Università degli Studi di Firenze, Sede "La Specola", Via Romana 17, Firenze, Italia

CLAUDIA CORTI

Museo di Storia Naturale dell'Università degli Studi di Firenze, Sede "La Specola", Via Romana 17, Firenze, Italia

In the last decades, the intensification of agricultural practices has caused huge and extremely rapid environmental changes. Reptiles often occur in agricultural landscapes and, due to their ecological features (i.e., limited dispersal ability and a mainly insectivorous diet), they face directly such transformation in terms of loss and alteration of habitats, of trophic resources, as well as direct physiological effects of agrochemicals. Working at field scale, we analyzed some ecological effects of agricultural management using the Italian Wall Lizard (*Podarcis siculus* Rafinesque, 1810) as model organism. Investigating the distribution of reptiles in an agricultural area in Italy, we found an overriding presence of *P. siculus*, the only species present in intensive crops (even if occurring just on the margins of arable fields). *P. siculus*, at least to a certain extent, is probably able to take advantage of the expansion of cultivated lands, open and often depleted areas where other species cannot persist. Examining *P. siculus* female breeding output in adjacent crops with different managements, we partly confirmed this hypothesis: we recorded the lowest breeding effort in almost unmanaged sites while egg mass, clutch mass, and relative fecundity increased in intensively managed sites. However, in the most intensive crops we detected a life-history trade-off between egg number and size, thus suggesting that intensive management imply some environmental constraints requiring a peculiar partitioning of breeding resources. Our results provide some basic ecological information that may be useful in the effort of developing efficient indicators for herpetofauna environmental risk assessments.

**EFFECTS OF PESTICIDES AND ANTIBIOTICS ON THE EARLY DEVELOPMENT OF THE MODEL AMPHIBIAN ORGANISM: *Xenopus laevis***

LAURENT BOUALIT

Institute of Earth Surface Dynamics, Faculty of Geosciences and the Environment, University of Lausanne, Lausanne, Switzerland.

HUGO CAYUELA

Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec City, Québec, Canada.

RAPHAEL SANTOS

HEPIA, University of Applied Sciences Western Switzerland, Ecology and Engineering of Aquatic Systems Research Group, Jussy, Switzerland.

NATHALIE CHEVRE

Institute of Earth Surface Dynamics, Faculty of Geosciences and the Environment, University of Lausanne, Lausanne, Switzerland.

With 40% of endangered species, amphibians represent one of the most threatened groups among vertebrates. Amphibian decline is multi-causal and pollution is usually considered as one of major factors involved in population decrease and local extinction. Especially, the use of pesticides and antibiotics in the context of farming activities is suspected to degrade the quality of breeding sites (e.g. lakes, swamp, and ponds).

In pond-breeding amphibians, embryonic and larval development takes place in aquatic habitats. Environmental conditions experienced over aquatic development are critical since they affect growth (rate and timing) and survival until metamorphosis. Therefore, exposure to chemicals during aquatic development may have a strong detrimental impact on amphibians' population dynamics.

To date, the impacts of pesticides and antibiotics on amphibians' populations are still poorly understood. To fill this gap, we aim at investigating the effects on embryos of African clawed frog (*Xenopus laevis*) of several pesticides and antibiotics regularly detected in worldwide surface waters. The main objective of this project is to identify and develop sensitive markers for highlighting effect of pesticides and antibiotics at early stages of amphibians' life-cycle. To improve individual monitoring, we adapted FETAX protocol relying on the Fish Embryo Acute Test (FET), primarily developed for zebrafish. Briefly, embryos are exposed to six concentrations over a 96h post fecundation (hpf) period and three apical observations (coagulation of embryos, lack of somite formation and lack of heartbeat) are recorded. On top of these observations, molecular biomarkers, embryos' activity and embryos' size are recorded as well.

**WHAT IS KNOWN AND WHAT IS STILL TO BE KNOWN ABOUT THE  
FUNCTION OF LIZARDS IN AGRO-ENVIRONMENTS**

MIGUEL A. CARRETERO

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

In a world undergoing a major biodiversity loss, conservation can no longer rely on recovering patterns but also on inferring the subjacent processes. Functional responses of organisms to disturbance need to be recognised, measured and predicted. Under multiple socioeconomic and environmental pressures, European agro-systems epitomize such conservation priorities and gaps. I will illustrate the approach focusing on lizards, pivotal elements in trophic webs, particularly in Mediterranean and insular agro-environments, where climate is less restrictive and other insectivorous vertebrates are less abundant. Ectothermy is advantageous in terms of metabolic cost but carries activity restrictions in space and time and exposure to water loss, radiation, competitors, parasites and predators. Balance between these, often conflicting, forces may be attained at different optima depending on species, population, sex, stage and season. Agricultural fields often harbour rich lizard assemblages depending on overlap opportunity and niche conservatism. However, there is a trend for niche shift, character displacement and community simplification with the spatio-temporal context being decisive. Flexibility in biological functions greatly vary across species either due to selection or to phenotypic plasticity. Species displaying lower variation tend to be restricted in habitat and distribution range. Resilience to disturbance (e.g. agrochemicals, spatial fragmentation and homogenisation) also relies on such flexibility with specialists becoming more threatened than generalists. Nevertheless, disturbance factors (climate change, pollution, habitat degradation, alien species) and biological responses (life history, thermal and water ecology) are interactive. This complex reality cannot be ignored when conserving agro-environments.

**POTENTIAL PESTICIDE EXPOSURE DURING THE POST-BREEDING  
MIGRATION OF THE COMMON TOAD (*Bufo bufo*) IN A VINEYARD  
DOMINATED LANDSCAPE**

CHRISTOPH LEEB

Institute for Environmental Sciences - University of Koblenz-Landau, 76829 Landau, Germany

CARSTEN BRÜHL

Institute for Environmental Sciences - University of Koblenz-Landau, 76829 Landau, Germany

KATHRIN THEISSINGER

Institute for Environmental Sciences - University of Koblenz-Landau, 76829 Landau, Germany

Two important factors for the global amphibian decline are habitat destruction due to an intensification of farming and a concordant increase of pesticide applications. Recent studies showed that there might be an underestimated risk of pesticide exposure for terrestrial amphibians. Moreover, there is little data on the terrestrial habitat use of amphibians in agricultural landscapes that allow an estimation of the exposure risk. To fill this knowledge gap we used telemetry and investigated the post-breeding migration of 51 common toads (*Bufo bufo*) from a breeding pond in a vineyard-dominated landscape in Southern Palatinate (Germany). In 15% of all relocations individuals were found directly in the vineyard. However, by comparing used and available habitats, our data suggest that toads tend to avoid vineyards as terrestrial habitats. To estimate a possible spatial-temporal overlap of toad migration and pesticide application we combined our telemetry data with information about pesticide applications. Seven individuals had a high probability (> 75%) of being directly exposed to a pesticide application. Taking spray drift and the half-life values of applied pesticides into account, the number raised to 15 individuals. We estimated that on a single day up to 24% of all toads came in contact with pesticides, resulting in a high overall exposure risk. Because pesticides can have negative effects on amphibians, we conclude that a heterogeneous cultural landscape with buffer strips around ponds, uncultivated patches and migration corridors might be the best management measure to sustain amphibians in the agricultural landscape.

**THE USE OF PLANT PROTECTION PRODUCTS AND ITS IMPACT ON  
REPTILES**

VALENTIN MINGO

Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany

STEFAN LÖTTERS

Trier University, Trier, Germany

NORMAN WAGNER

Trier University, Trier, Germany

Habitat loss and environmental pollution are among the main causes responsible for worldwide biodiversity loss. The resulting species and population declines affect all vertebrates including reptiles. Especially in industrialized countries, pollution by agrochemicals is of remarkable importance. Here, habitat loss has historically been associated with expansion of agriculture. Species persisting in such environments do therefore not only need to cope with habitat loss, but more recently, also with chemical intensification, namely pesticide exposure. However, reptiles are currently not considered in risk assessments during the admission of plant protection products. A Scientific Opinion on the state of the science on pesticide risk assessment for amphibians and reptiles was recently published by the EFSA PPR-Panel. Here, the coverage of the risk to amphibians and reptiles by current risk assessments for other vertebrate groups was investigated. The authors concluded that data, especially for reptiles, is greatly lacking. In an aim to study the potential effects pesticide exposure on wild reptile populations, we studied different common wall lizard populations occurring in agricultural landscapes (vineyards) with regards to sub-individual (enzymatic biomarkers), individual (fitness) and population level (population structure) end points. Our results demonstrate a significant impact on these endpoints. Lab trials using conventional field doses of frequently applied pesticide formulations further resulted in impairments of locomotor performance, often coupled with fever responses. We thus conclude that reptiles do indeed suffer from negative effects resulting from exposure to conventional pesticides and should be included in ecotoxicological risk assessments for the admission of plant protection products.

**TOWARDS A GUIDANCE DOCUMENT ON RISK ASSESSMENT FOR  
AMPHIBIANS AND REPTILES: TELEMETRY DATA OF SAND LIZARDS (*Lacerta  
agilis*) TO RECORD FIELD DATA RELEVANT TO REGISTRATION**

GIANPAOLO MONTINARO

Rifcon GmbH, D-69493 Hirschberg, Germany

JAN-DIETER LUDWIGS

Rifcon GmbH, D-69493 Hirschberg, Germany

Currently Environmental risk assessments (ERA) for amphibians and reptiles are still considered to be covered by ERAs for birds and mammals. The recent Scientific Opinion on the state of the science on pesticide RA for amphibians and reptiles highlights the need to create specific RA schemes for amphibians and reptiles. However, as in ERAs for birds and mammals, radio-tracking studies are performed to calculate so-called 'PT factors' reflecting 'proportion of an animal's daily diet obtained in treated fields'. Since it is assumed that the percentage of ingested food is equivalent to the time an individual spends actively within a crop, such telemetry data can be used to define exposure or the proportion of treated diet. EFSA (2018) recommends collecting such radio-tracking data for amphibians and reptiles as well. We will briefly introduce into the current ERA scheme for birds & mammals with available data focusing on the PT factor, and show preliminary data of a pilot study started in 2018 in an agricultural landscape with a predominance of vineyards. The aim of the study is to test the feasibility of tracking adult specimen continuously from sunrise to sunset as recommended by EFSA using different devices and assess the applicability of collaring and radio-tracking method. Sand lizards are indicated as a potential 'Focal Species' lizard for ERA. The result obtained at the end of the reproductive period (July-August) will be presented and discussed: daily activity, habitat use and PT factors.

**IMPROVING EFFECTIVENESS OF PESTICIDE RISK ASSESSMENT FOR  
AMPHIBIANS AND REPTILES**

MANUEL E. ORTIZ-SANTALIESTRA

Spanish Institute of Game and Wildlife Research (IREC) UCLM-CSIC-JCCM, Ciudad Real, Spain

Prospective risk assessment of pesticides is meant to guarantee that no unacceptable risks to wildlife are associated with the environmental release of these substances. Because amphibians and reptiles have been traditionally neglected in pesticide risk assessment, the extent at which this procedure is useful to protect herpetofauna from pesticide impacts is uncertain. Several research actions and evaluations of the state of the science have been conducted over recent years in order to elucidate the degree of protection conferred by current risk assessment procedures to herpetofauna and propose possible solutions. The leading action was the Scientific Opinion published by the European Food Safety Authority (EFSA), proposing a risk assessment scheme for amphibians and reptiles and suggesting knowledge gaps that need to be addressed at the short or medium term. Available information reflects a general lack of protection provided by current pesticide risk assessment scheme to amphibians and reptiles, but information retrieved from other vertebrates relative to specific substances or exposure routes can be used in order to avoid an unnecessary use of animals for regulatory purposes. Solutions are proposed to address knowledge gaps using alternative methods, like individual-based models, that help improving ecological relevance of pesticide risk assessment for amphibians and reptiles without necessarily adding animal testing to the regulated procedures. This presentation will summarize those proposed solutions, enhancing the multidisciplinary aspects of the actions that will have to be taken in upcoming years, and focusing in the key role that herpetologists' community should play in this challenge.



**MULTI-DISCIPLINARY APPROACH TO ASSESS PESTICIDES EFFECTS ON  
LIZARDS (*Podarcis siculus*)**

GIULIA SIMBULA

Dipartimento di Scienze, Università Roma Tre, Rome, Italy

GINEVRA MOLTEDO

ISPRA, Rome, Italy

BARBARA CATALANO

ISPRA, Rome, Italy

GIACOMO MARTUCCIO

ISPRA, Rome, Italy

CLAUDIA SEBBIO

ISPRA, Rome, Italy

FULVIO ONORATI

ISPRA, Rome, Italy

LEONARDO VIGNOLI

Dipartimento di Scienze, Università Roma Tre, Rome, Italy

Extensive use of pesticides may have drastic effects on non-target species, as amphibians and reptiles. In our work, we selected the Italian wall lizard *Podarcis siculus* as a model species to investigate if and how two different types of field treatment in nine areas in central Italy affected the health status of individuals. We performed analyses by means of a multi-disciplinary approach considering body condition, parasite load, biomarkers such as (i) AChE activity in the nervous system, (ii) DNA damage measured in the erythrocytes, (iii) oxidative stress (TOSCAssay toward peroxy radicals) and biotransformation enzymes (GSTs) in the liver. Our results showed no significant alterations for the AChE activity and the antioxidant system among treatments, whereas an increase of micronuclei formation was reported in one conventional site. As for the parasite load, a higher prevalence of parasites with indirect life cycle (insects as first host) was found in lizards from organic fields. The AChE and GSTs are enzymes representing an early toxicity response to pollutants and the MN has been described as a sort of repository of past damage events. The lack of antioxidant system's alteration suggests that genotoxicity effect of pesticides did not depend on peroxy radicals formation. Further analyses are needed to better understand if the absence of significant responses were due to a tolerance mechanism developed by chronic exposure, or other mechanisms were involved in the toxicity of pesticides for this species.



# Workshop: Herp hybrid zones

**DIFFERENTIAL INTROGRESSION ACROSS NEWT HYBRID ZONES –  
EVIDENCE FROM REPLICATED TRANSECTS**

PIOTR ZIELIŃSKI

Institute of Environmental Sciences, Jagiellonian University Kraków, Poland

KATARZYNA DUDEK

Institute of Environmental Sciences, Jagiellonian University Kraków, Poland

GEMMA PALOMAR

Institute of Environmental Sciences, Jagiellonian University Kraków, Poland

WIESŁAW BABIK

Institute of Environmental Sciences, Jagiellonian University Kraków, Poland

Examination of genome-wide patterns of introgression is crucial to understand the genomic architecture of speciation. Hybrid zones allow for direct quantification of introgression under natural conditions. Because the genetic basis of reproductive isolation may differ across species range and introgression can be affected by environmental factors, the genomic landscape of introgression should be assessed in multiple transects throughout the hybrid zone. However, such studies remain rare. Here, we analyse introgression in replicated transects through two hybrid zones between the Carpathian newt (*Lissotriton montandoni*) and two evolutionary lineages of the smooth newt (*L. vulgaris ampelensis* and *L. v. vulgaris*). More than 1000 protein coding genes located in a linkage map were examined in 154 populations from 5 transects to test for differences in genome-wide landscape of introgression between the hybrid zones. Narrow allele frequency clines and bimodally distributed genotypes in mixed populations indicate strong reproductive isolation in all transects. However we detected marked differences in the extent of hybridization and introgression between the two hybrid zones. These differences result probably from different ages of the two zones, as suggested by the differences in the length of genomic tracts of introgression. The genomic landscape of introgression did not differ between zones more than between transects within zones, but was heterogeneous - genes showing increased introgression were overrepresented on two out of 12 linkage groups. Our results demonstrate that the genomic landscape of introgression can differ in various regions of the contact zone even at late stages of speciation.

**ALTERNATIVE OUTCOMES OF HYBRIDIZATION IN WATER FROGS (GEN. *Pelophylax*) REVEALED BY IN VITRO CROSSINGS AND MOLECULAR APPROACHES**

ADRIANA BELLATI

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

SUSANNA SEGHIZZI

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

ALESSANDRO BOLIS

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

CARLOTTA RAFFA

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

HELEN GOMIS

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

ANTONIETTA GROSSO

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

DANIELE DELLE MONACHE

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

The genus frog *Pelophylax* Fitzinger, 1843 has been widely introduced in Europe and now threatens the persistence of local biota via predation, competition, spread of pathogens and genomic introgression. In populations where multiple lineages have been repeatedly introduced, hybridization may occur either with native species or between different alien taxa, as a result of labile reproductive barriers and hybridogenetic mechanisms. The outcomes of such reproductive interactions are still largely unknown in terms of loss of genetic diversity, genomic introgression and hybrid performance. To assess potential impacts of alien genome introgression on the native hybridogenetic system of *P. lessonae* and *P. kl. esculentus* in northern Italy, we coupled laboratory experiments and wild populations' surveys by performing i) in vitro crossings of pure parental lineages detected via diagnostic molecular markers (*P. lessonae*/*P. kl. esculentus* vs. *P. ridibundus*, *P. kurtmuelleri*, and *P. bedriagae s.s.*), ii) hybrids breeding until reproduction under controlled conditions, and iii) molecular characterization of F1 tadpoles. Results show significant variation in the offspring stemming from different parental crossings in development, post-metamorphic survival, fertility, and genetic variability, suggesting alternative drawbacks of hybridization and hybridogenetic mechanisms for the persistence of native water frog systems in northern Italy. This research was supported by the SEH Grant in Herpetology – Conservation.

**TRANSCRIPTOME BASED SNP MARKERS REVEAL DYNAMICS OF THE  
MARBLED NEWTS' HYBRID ZONE**

JULIA LOPEZ DELGADO  
Leiden University, Leiden, The Netherlands

Hybrid zones represent ideal natural laboratories for unravelling the spatial and temporal dynamics of species ranges. Secondary contact between species that underwent allopatric speciation in Pleistocene glacial refugia often results in the formation of hybrid zones, allowing for interspecific flow among the taxa. The movement of hybrid zones results from the displacement of one species by another. Hybrid zone movement has been suggested for the marbled newts in the Iberian Peninsula, based on mitochondrial and nuclear data. The analysis of the genetic footprint across the Lisbon Peninsula, southwest of the contact zone between *Triturus pygmaeus* and *T. marmoratus*, provides a suitable scenario for testing the dynamic nature of the hybrid zone. One mitochondrial and 54 nuclear genetic markers across 32 populations were employed to test for a genetic spatial signature of hybrid zone movement. The existence of a previously described *T. marmoratus* enclave in northwestern Lisbon Peninsula was further evidenced, but no molecular evidence of a *T. marmoratus* genetic footprint across the landscape was found. Plausible scenarios resulting in the reported genetic patterns among the marbled newts are investigated.

**PERVASIVE INTROGRESSION OF MHC GENES IN NEWT HYBRID ZONES**

KATARZYNA DUDEK

Jagiellonian University, Institute of Environmental Sciences, Kraków, Poland

TOMASZ GACZOREK

Jagiellonian University, Institute of Environmental Sciences, Kraków, Poland

PIOTR ZIELIŃSKI

Jagiellonian University, Institute of Environmental Sciences, Kraków, Poland

WIESŁAW BABIK

Jagiellonian University, Institute of Environmental Sciences, Kraków, Poland

Variation in the vertebrate major histocompatibility complex (MHC) genes plays a crucial role in fighting pathogen assault, making MHC a prime target of balancing selection. Because new alleles confer selective advantage, MHC should introgress easily even under limited hybridization. Using replicated transects through hybrid zones between strongly reproductively isolated newt species (*Lissotriton montandoni* and *L. vulgaris*), we demonstrated recent or ongoing MHC introgression. In one zone, MHC similarity between species within transects exceeded that between transects within species, implying pervasive introgression – a massive exchange of MHC genes, not limited to specific variants. In simulations, the observed pattern was produced under the combined action of balancing selection and hybridization, but not when these processes acted separately. Thus, massive introgression at advanced stages of divergence can introduce new and restore previously lost MHC variation, boosting the adaptive potential of hybridizing taxa. In consequence, MHC genes may be the last to stop introgressing between incipient species. This finding has implications for design and implementation of conservation strategies in the age of the global amphibian crisis.

## HYBRID ZONE GENOMICS OF EUROPEAN ANURANS

CHRISTOPHE DUFRESNES

Department of Animal & Plant Sciences, University of Sheffield, United Kingdom

SPARTAK LITVINCHUK

Zoological Institute of St Petersburg, Russian Academy of Sciences, St Petersburg, Russia

INIGO MARTINEZ-SOLANO

Museo Nacional de Ciencias Naturales, Madrid, Spain

NICOLAS PERRIN

Department of Ecology & Evolution, University of Lausanne, Switzerland

ROGER BUTLIN

Department of Animal & Plant Sciences, University of Sheffield, United Kingdom

PIERRE-ANDRE CROCHET

Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), CNRS, Montpellier, France

The multiple hybrid zones formed between closely-related anurans in Europe offer natural laboratories to gauge reproductive isolation between diverging lineages *in statu nascenti*. Through genome-wide analyses (RAD-seq), we surveyed eight pairs of nascent species representing all anuran families present in Europe and hybridizing in natural contact zones, i. e. *Alytes obstetricans pertinax* / *almogavarii* (Alytidae), *Rana temporaria temporaria* / *parvipalmata* (Ranidae), *Bufo bufo* / *spinus* (Bufonidae), *Hyla intermedia* / *perrini* (Hylidae), *Pelobates fuscus* / *vespertinus* (Pelobatidae), *Bombina bombina* / *variegata* (Bombinatoridae), *Pelodytes ibericus* / *atlanticus* and *P. punctatus* / *hespericus* (Pelodytidae). We found average hybrid zone widths to be irrespective of divergence time but rather reflected system-specific and local hybrid zone dynamics. Some transitions were asymmetric and may be affected by intrinsic features like hybrid zone movement and heterogametic transitions in sex-determination systems. As reproductive isolation accumulates between cryptic species pairs, locus-specific responses become increasingly heterogeneous (with a bimodal distribution of cline width), illustrating how genetic incompatibilities progressively affect some loci more than others, not the whole genome at once. Future work will aim at contrasting the genomic location of these two types of loci. Altogether, our study provides a framework to understand how the genetic architecture of post-zygotic isolation builds up in space and time and further contributes to the debated delimitation of cryptic taxa.



**DEEP BRANCH GENEFLOW IN A HYBRIDIZATION RICH DIVERSIFICATION  
PROCESS**

SUSANA FREITAS

University of Lausanne, Lausanne, Switzerland

D. JAMES HARRIS

CIBIO-INBIO, Porto, Portugal

MARINE ARAKELYAN

University of Yerevan, Yerevan, Armenia

MIGUEL CARRETERO

CIBIO-INBIO, Porto, Portugal

ROGER BUTLIN

University of Sheffield, Sheffield, United Kingdom

Hybridization between different populations has been considered to be mostly ineffective, hybrid zones expected to be transient and evidence of reproductive barriers between diverging groups. With the development of high-throughput sequencing, extensive evidence has been provided of ongoing introgressive hybridisation within and between diverging clades. In vertebrates, interspecific hybridization is the most common process responsible for the origin of new parthenogenetic species. However, it is not known how the hybrid origin of parthenogenesis fits the overall history of hybridization within a given group. In this study we assess the extent of introgressive hybridization and secondary contact that *Darevskia* sexual species underwent during divergence. This genus of rock lizards is found in the Caucasus and presents sexual and asexual species, the later resulting from the recent (~100 kyrs) hybridization between a specific sexual species pair. We used targeted re-sequencing to analyse over 600 loci in a phylogenetic framework. We found widespread evidence of introgressive hybridization in *Darevskia*, between both recent and more anciently diverged taxa. However, despite the widespread gene flow detected, no evidence of gene flow was found between the parental species of the asexual hybrids. When these species hybridized and originated the asexual hybrids, gene flow was most likely prevented by reproductive barriers between the species pairs. This study highlights the evidence for hybrid asexuality acting as a reproductive isolation barrier that arises before other pre-zygotic mechanisms, and the specificity of the conditions that surround the origin of hybrid asexuality in vertebrates, contributing to its low frequency in nature.

**SLOW-WORM LIZARDS (*Anguis*) – A NEW REPTILE MODEL IN HYBRID ZONE STUDIES**

VACLAV GVOZDIK

Institute of Vertebrate Biology of the Czech Academy of Sciences, Brno, Czech Republic

STUART J.E. BAIRD

Institute of Vertebrate Biology of the Czech Academy of Sciences, Brno, Czech Republic

DANIEL JABLONSKI

Comenius University in Bratislava, Faculty of Natural Sciences, Department of Zoology, Bratislava, Slovakia

DAVID JANDZIK

Comenius University in Bratislava, Faculty of Natural Sciences, Department of Zoology, Bratislava, Slovakia

Charles University, Faculty of Science, Department of Zoology, Prague, Czech Republic

PETER MIKULICEK

Comenius University in Bratislava, Faculty of Natural Sciences, Department of Zoology, Bratislava, Slovakia

JIRI MORAVEC

National Museum, Department of Zoology, Prague, Czech Republic

Slow worms (*Anguis*) are legless lizards (Anguidae) distributed across the western Palearctic, predominantly in Europe. Five species with mostly parapatric distributions, whose ranges form four secondary contact zones, are currently recognized. Two species, *A. fragilis* and *A. colchica*, are widespread in western and eastern Europe, respectively. Their current ranges form a long secondary contact zone running from the Baltic region, down through central Europe to the north-eastern Balkans. This contact zone represents in reptiles a unique, more than 2000 km long natural laboratory to study microevolutionary processes, which form barriers maintaining divergence between species despite gene flow. Our project aims to study this model system in central Europe to understand levels of gene flow on a genome-wide scale, and to test association between genomic variation and environmental parameters. This will allow us to assess if the two slow-worm species respond to environmental factors in a similar or different way, and to understand the balance between endogenous and exogenous selection acting in the contact zones.

**HABITAT SELECTION AND MORPHOLOGICAL VARIATION ACROSS HUMAN-ALTERED HABITATS IN SECONDARY CONTACT ZONES: THE CASE OF WESTERN MEDITERRANEAN VIPERS IN NORTHERN IBERIA**

INÊS FREITAS

CIBIO-InBIO, University of Porto, Portugal

ÓSCAR ZUAZO

Cl. La Puebla, 1. 1ªA. 26250 Santo Domingo de la Calzada, La Rioja, Spain

PEDRO TARROSO

CIBIO-InBIO, University of Porto, Portugal

ANTIGONI KALIONTZOPOULOU

CIBIO-InBIO, University of Porto, Portugal

FERNANDO MARTÍNEZ-FREIRÍA

CIBIO-InBIO, University of Porto, Portugal

Anthropogenic landscape transformation imposes novel selective forces on natural populations, potentially causing changes in species requirements, behaviour and morphology. Yet, its impact over species dynamics and how it shapes trait evolution in coexistent species remain poorly explored. Here, we investigate the role of climatic variability and landscape transformation in shaping patterns of habitat selection and morphological variability of Western Mediterranean vipers (*Vipera aspis* and *V. latastei*), across two contact zones in Northern Iberia characterized by contrasting levels of human disturbance: the High Ebro (a natural landscape) and Oja-Tiron (an intensive cereal culture landscape). Ecological niche models (ENM) were performed for each species in each contact zone (1037 1x1km records), using a set of topo-climatic and habitat variables. Multivariate analyses (discriminant, variation and trajectory analyses) were performed over ten phenotypic traits for ca. 1000 specimens from both species and contact zones. ENMs revealed similar topo-climatic correlates for the distribution of each species in each contact zone. However, habitat correlates for each species greatly varied when contact zones were compared, suggesting that species are forced to inhabit contrasting structural areas. Discriminant analyses showed higher power to differentiate species in Oja-Tiron, suggesting that increased competition may have promoted higher species differentiation (i.e. character displacement). Additionally, ANOVA and trajectory analyses show clear differences between species and sexes in the patterns of morphological variation, suggesting different responses to human disturbance. Overall, our results provide evidence for the influence of human disturbance on the patterns of phenotypic variation and habitat selection of these vipers.

**SPATIAL VARIATION IN INTROGRESSION ALONG THE COMMON TOAD  
HYBRID ZONE**

ISOLDE VAN RIEMSDIJK

Naturalis Biodiversity Center, Leiden, The Netherlands

JAN W. ARNTZEN

Naturalis Biodiversity Center, Leiden, The Netherlands

GARY BUCCHIARELLI

University of California, Los Angeles, California

EVAN MCCARTNEY-MELSTAD

University of California, Los Angeles, California

MARINA RAFAJLOVIC

University of Gothenburg, Gothenburg, Sweden

PETER SCOTT

University of California, Los Angeles, California

ERIN TOFFELMIER

University of California, Los Angeles, California

BRAD SHAFFER

University of California, Los Angeles, California

BEN WIELSTRA

Leiden University, Leiden, The Netherlands

Individual barrier genes restrict gene flow between populations. Many barrier genes together can form a strong barrier to gene flow (barrier effect), ultimately leading to complete reproductive isolation. Transitions of barrier genes are steep compared to neutral genes, and are restricted to be located together in the hybrid zone. When patterns of restricted gene flow are consistent throughout a hybrid zone, this points to a universal (evolutionary) cause of the barrier effect. In France, a hybrid zone between two toad species, *Bufo bufo* and *B. spinosus*, shows asymmetric introgression. We use ~ 1200 SNPs to compare patterns of introgression for two transects, one in northwestern France and one in southeastern France. In the northwest transect, introgression of neutral markers from *B. spinosus* into *B. bufo* coincides with a northward shift in the peak of admixture linkage disequilibrium, which is most consistent with asymmetric reproductive isolation. In the southeast transect, introgression is symmetric and a peak of admixture linkage disequilibrium coincides with the hybrid zone center, consistent with a stable hybrid zone. Remarkably, the barrier effect appears to be twice as strong in southeast than in northwest France. Overlap of barrier markers occurring in both transects is high, suggesting a universal barrier effect exists along the hybrid zone. The involvement of distinct genetic groups on the *B. bufo* side of the zone best explains these results. The common toad hybrid zone is more complex than previously appreciated.

**UNRAVELLING THE GEOGRAPHICAL PATTERNS OF INTROGRESSION IN  
WALL LIZARDS USING LANDSCAPE GENTICS**

MARAVILLAS RUIZ MINANO

Tasmania university, hobart, tasmania

lund university, lund, sweden

TOBIAS ULLER

Lund university, lund, sweden

GEOFF WHILE

Tasmania univresity, hobart, tasmania

Increasing evidence suggests that phenotypic variation in both plants and animals can be the result of genetic exchange between species and subspecies. The rate and direction of genetic exchange will be strongly influenced by patterns of selection and thus mediated by the biotic and abiotic environment. One result of this is a distinctive pattern of genetic exchange across the landscape, making secondary contact zones excellent settings to investigate ongoing selection processes in the wild. Here I examine how aspects of the environment mediate gene flow upon secondary contact in the common wall lizard, *Podarcis muralis*. This species exhibits considerable phenotypic and genetic divergence across its species range with distinct evolutionary lineages having back into secondary contact in northern Italy. We have previously shown strong asymmetric introgression of genes between lineages in these zones of secondary contact driven by sexual selection. However, this introgression appears to be limited at high altitudes. I will present preliminary analysis of the climatic factors that mediate gene flow within these regions. This includes a large data set including phenotypic data from over 170 populations spanning the altitudinal and geographic range of the hybrid zone. These data suggest that climatic variables have an effect on introgression of the phenotypic traits across the landscape. I will discuss these results within the context of the selective mechanisms that may link climate to limited gene flow.

**THE EVOLUTIONARY CONSEQUENCES OF LOCAL ADAPTATION AND  
HYBRIDIZATION IN THE WORLDS' ONLY SEA GOING LIZARD - THE  
GALÁPAGOS MARINE IGUANA**

SEBASTIAN STEINFARTZ

University of Leipzig, Molecular Evolution and Systematics of Animals, Leipzig, Germany

AMY MACLEOD

University of Leipzig, Molecular Evolution and Systematics of Animals, Leipzig, Germany

STEN ANSLAN

Technische Universität Braunschweig, Department of Evolutionary Biology, Braunschweig, Germany

TIMM REINHARDT

University of Leipzig, Molecular Evolution and Systematics of Animals, Leipzig, Germany / Federal Agency for Nature  
Conservation, Bonn, Germany

JUAN M. GUAYASAMIN

Colegio de Ciencias Biológicas y Ambientales (COCIBA), Universidad San Francisco de Quito, Ecuador

DIEGO PÁEZ-ROSAS,

Colegio de Ciencias Biológicas y Ambientales, COCIBA, Universidad San Francisco de Quito, Ecuador

NICOLÁS PEÑAFIEL

Colegio de Ciencias Biológicas y Ambientales (COCIBA), Universidad San Francisco de Quito, Ecuador

Local adaptation and hybridization have been long seen as contrasting evolutionary processes. In general, gene flow will counteract evolutionary adaptation by breaking up the genetic architecture of genes underlying adaptive evolutionary traits. Hybridization, which can be seen as a special form of gene flow between distinct species and genetically differentiated lineages or populations, should be therefore counteracting evolutionary adaptation and speciation. However, recently hybridization has gained much attention as an important evolutionary force that is acting in natural populations. Here, we present a natural system, the Galápagos marine iguanas, in which local adaptation/incipient speciation is paralleled by inter-island hybridization. We hypothesize that the combination of both processes – local adaptation and hybridization - results in the integration of the genomic equivalents of adaptations on a local level into a common species gene pool by recurrent hybridization events. Such a mechanism could increase the adaptive potential of populations and of the species as a whole, thus enhancing its evolutionary potential.

**WHAT *Triturus* NEWTS CAN TELL US ABOUT DIVERSIFICATION AND EVOLUTION WITHIN HYBRID ZONE?**

TIJANA VUČIĆ

Faculty of Biology University of Belgrade, Belgrade, Serbia  
Institute for Biological Research “Siniša Stanković” University of Belgrade, Belgrade, Serbia

MILENA CVIJANOVIĆ

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

ANA IVANOVIĆ

Faculty of Biology University of Belgrade, Belgrade, Serbia

The natural hybrid zone of *Triturus ivanbureschi* and *T. macedonicus* in the central Balkan Peninsula with a specific species displacement scenario provides an excellent background for evolutionary studies. We set up a common garden experiment for breeding and reciprocal crossing of these two species from populations out of the hybrid zone. We collected data on life-history and morphological diversification throughout ontogeny. Our results showed that there are no pre- or postzygotic reproductive barriers, with proven fecundity of F1 generation in all crossings. Reproductive characteristics and survival rates were similar for both species and their hybrids. Hybridization significantly affects morphological variation, with hybrids showing distinct tail and head morphology compared to parental species. The head shape ontogeny from hatchling to metamorphosed stage was used to explore postembryonic ontogenetic trajectories and to test whether metamorphosis acts as developmental constraint. Differences in the developmental rate of the two species were found. Hybrids had intermediate values relative to parental species. Also, obtained results revealed that metamorphosis cannot be regarded as a developmental constraint for salamander head shape. Overall, results obtained from the common garden experiment provided an insight into evolutionary mechanisms that lead to divergence from the common ancestral developmental program and evolution of ontogenies in the hybrid zone.

## **HYBRID ZONE MOVEMENT IN CRESTED NEWTS**

BEN WIELSTRA

Institute of Biology Leiden, Leiden University, Leiden, The Netherlands

Speciation typically involves a stage in which species can still exchange genetic material. Interspecific gene flow is facilitated by the hybrid zones that such species establish upon secondary contact. If one member of a hybridizing species pair displaces the other, their hybrid zone would move across the landscape. Such movement has occasionally been observed over years or even decades. This suggests that hybrid zones have the potential to traverse considerable distances over evolutionary time. Yet, the prevalence of such long-term hybrid zone movement is poorly understood. A key prediction of hybrid zone movement is that the receding species leaves behind a trail of introgressed selectively neutral alleles within the expanding one. We test for such a genomic footprint of hybrid zone movement in two hybrid zones between crested newt species (genus *Triturus*) that are thought to have shifted position. The strongly asymmetrical and geographically extensive introgression we uncover in the two crested newt cases provide firm support for hybrid zone movement proceeding over considerable time and space.



**GENOMIC PATTERNS OF PARALLEL HYBRID ZONES PROVIDE INSIGHT  
INTO THE CAUSES OF INTROGRESSION AND SPECIATION IN COMMON  
WALL LIZARD**

WEIZHAO YANG

Department of Biology, Lund University, Lund, Sweden

TOBIAS ULLER

Department of Biology, Lund University, Lund, Sweden

Speciation is the result of accumulation of reproductive barriers between populations, but establishing how these barriers arise is often difficult. Hybrid zones can form when two lineages come into contact and the barriers between them are still incomplete, which offer us the opportunity to study the mechanism of speciation. We examined two parallel hybrid zones between two divergent lineages in common wall lizard, *Podarcis muralis*, and took advantage of double-digest RAD sequencing to investigate how these hybrid zones formed, and reveal the genomic patterns over the hybrid zones. First, population structure analysis and demographic simulation showed that both hybrid zones were established via recent secondary contact by two highly divergent lineages. Second, Bayesian genomic cline model suggests that selection has promoted introgression of several loci from one lineage into the other, while introgression is highly limited for other loci. The introgressed loci showed limited concordance in the two hybrid zones, partly as a result of phenotypic differences within one of the lineages. The loci with lower-than-expected introgression that were shared between hybrid zones were associated with a number of candidate genes, which point towards putative biological mechanisms behind the speciation process.



# Workshop: Islands: from the Sea to the Sky

**ISLAND EFFECT ON INTRASPECIFIC AND INTERSEXUAL MORPHOLOGICAL  
VARIABILITY IN *Podarcis siculus***

VASCO AVRAMO

Università “ La Sapienza” di Roma

CLAUDIA CORTI

Museo “La Specola” di Firenze

GABRIELE SENCZUK

Museo “La Specola” di Firenze

WOLFGANG BÖHME

Zoologisches Forschungsmuseum Alexander Koenig

RICCARDO CASTIGLIA

Università “ La Sapienza” di Roma

PAOLO COLANGELO

Consiglio Nazionale delle Ricerche

Islands represent an important source of information for evolutionary biologists. Several studies have shown that prolonged isolation and particular environmental conditions can induce remarkable phenotypic diversification, even on sexual dimorphism. Indeed, in dimorphic species, an important component of the intraspecific phenotypic variability is due to differences between sexes, and the degree of these differences (sexual dimorphism) can be influenced by many ecological factors (food availability, demography, quality of resources), which can considerably vary in insular contexts. In our study, we explored whether there is an “islands effect” that could have influenced the morphological variability and the sexual dimorphism in the Italian wall lizard (*Podarcis siculus*). A Geometric Morphometrics approach was performed to analyze the morphological (head shape and size) variation of 27 Italian island populations and 15 continental ones, for a total of 720 samples, in order to test (1) whether island populations show a stronger higher morphological diversification (disparity) with respect to the continental populations, and (2) whether sexual dimorphism gets altered in islands contexts. Our results, although preliminary, show an increased shape variance, in both males and females from island populations, but no differences in size disparity were detected. Sexual dimorphism seems to be constantly lower on islands, both for shape and size, although no statistical significance was observed.

**DALMATIAN ODDITIES: FUNCTIONAL DIVERGENCE OF HEAD SHAPE IN LIZARDS ON TWO PECULIAR ISLANDS IN THE ADRIATIC**

SIMON BAECKENS

University of Antwerp, Wilrijk, Belgium

WANNES LEIRS

University of Antwerp, Wilrijk, Belgium

RAOUL VAN DAMME

University of Antwerp, Wilrijk, Belgium

Animals on islands are often morphologically divergent from their conspecifics on the mainland or on neighbouring islands as a result of selection favouring a specific ‘optimal’ phenotype for efficient resource acquisition in the unique insular environment they inhabit. To understand the adaptive significance of the observed phenotypic divergence on islands, it is crucial to have knowledge on the relationship between morphology and function.

The Dalmatian wall lizard (*Podarcis melisellensis*) is one of the most abundant members of the Adriatic insular herpetofauna. Although the general morphology of *P. melisellensis* lizards is relatively conservative, recent observations indicate that on two aberrant Adriatic islets (Brusnik and Mali-Barjak), lizards seem to deviate substantially in head shape from conspecifics on islands nearby. We predict that the observed divergence in head morphology is functionally significant, as a particular head shape allows lizards to feed more efficiently on the prey available. Indeed, diet analyses and estimates of invertebrate abundance showed that lizards on Mali-Barjak fed on smaller prey items than lizards on Brusnik as a consequence of differences in food availability. Moreover, geometric morphometric analyses of the head indicated that lizards on Brusnik had a short, wide head that enables them to bite hard and crush large prey, while Mali-Barjak lizards had a long, narrow head and low bite force. Lastly, behavioural assays of feeding performance showed that lizards from both islands fed most efficiently on prey that was available on their home islands, providing convincing evidence for functional divergence of head shape in *P. melisellensis*.

**TRAIT CHANGES IN *Podarcis erhardii* FROM A REPLICATED ISLAND  
INTRODUCTION EXPERIMENT IN GREECE**

COLIN DONIHUE

Department of Biology, Washington University, St. Louis, Missouri, USA

ANTHONY HERREL

UMMR 7179 CNRS/MNHN, Paris, France

MENELIA VASILOPOULOU-KAMPITSI

Department of Biology, University of Antwerp, Wilrijk, Belgium

ANNE-CLAIRE FABRE

Department of Life Sciences, The Natural History Museum, London SW7 5DB, United Kingdom

JOHANNES FOUFOPOULOS

School for Environment and Sustainability, University of Michigan, Ann Arbor, USA

PANAYIOTIS PAFILIS

Department of Biology, National and Kapodistrian University of Athens, Athens Greece.

Adaptive trait plasticity is widespread and can both facilitate survival in novel environments and serve as a precursor to subsequent evolution. We investigated changes in a suite of ecologically important traits following the experimental introduction of *Podarcis erhardii* lizards to five Greek islets. Here we demonstrate that in the founding population body condition, not absolute bite force, predicted initial survival and greater bite force plasticity predicted long-term survival. In the intervening five years, mean lizard densities and scars from intraspecific wounds became significantly more prevalent. Concurrently, absolute bite force became a significant predictor of subsequent lizard body condition and survival and female bite force allometry significantly increased to match that of males. We also report differences in thermal ecology, and chemical signal design. Overall, we found that flexible phenotypic expression in response to novel environmental conditions can have important and immediate survival consequences and subsequent long-term impacts on the evolutionary trajectory of a species.

**LIZARDS ON ISLANDS AS MODELS FOR RAPID ADAPTIVE DIVERSIFICATION**

ANTHONY HERREL

CNRS/MNHN, Paris, France

COLIN DONIHUE

Washington University St. Louis

JONATHAN LOSOS

Washington University St. Louis

Island lizards are often characterized by differences in morphology compared to their mainland counterparts. Although previous studies have documented differences in body size, head morphology, bite force and diet in insular environments, the selective pressures driving the evolution of this insular phenotype remain poorly known. Moreover, how fast lizards evolve these insular phenotypes has never been assessed. Here we provide data for lizards from insular ecosystems (*Podarcis* and *Anolis*) documenting the possible drivers of the observed phenotypic differences in insular lizards. Additionally, we provide data on how fast these phenotypes may evolve. Our results show that drivers of phenotypic variation are related to island size, food availability, population density as well as founder effects. Our results show that these changes occur much faster than what is typically thought and suggest an important role for phenotypic plasticity.

**POPULATION GENETICS OF THE ANDAMAN DAY GECKO (*Phelsuma andamanensis*; BLYTH,1861) ON THE ANDAMAN ISLANDS; AN IDIOSYNCRATIC SPECIES OF UNIQUE BIOGEOGRAPHIC ORIGINS BUT LIMITED GENETIC STRUCTURE**

ASHWINI VENKATANARAYANA MOHAN

Zoological Institute, Department of Evolutionary Biology, Braunschweig University of Technology, Braunschweig, Germany

PABLO OROZCO TER WENGEL

School of Biosciences, Cardiff University, Cardiff, Wales, United Kingdom

KARTIK SHANKER

Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India

MIGUEL VENCES

Zoological Institute, Department of Evolutionary Biology, Braunschweig University of Technology, Braunschweig, Germany

The Andaman and Nicobar archipelago are continental islands in the Bay of Bengal. The Andaman day gecko (*Phelsuma andamanensis*) is endemic to the Andaman Islands, but its ancestors originated several thousand kilometers away in Madagascar. *Phelsuma* geckos have diversified on almost all the islands they have colonized, and the genus is exclusive to the West Indian Ocean Islands, except the Andamans. To test diversification of the Andaman day gecko, we extracted DNA from 141 individuals sampled on 9 islands. We then amplified 13 specifically designed microsatellite markers and sequenced a segment of the mitochondrial gene Cytochrome C Oxidase I (COI). To compare levels of genetic differentiation between island populations of Gekkonidae, we further sequenced a segment of the 16S rRNA gene from individuals of the Andaman day gecko, the Andaman bent-toed gecko (*Cyrtodactylus rubidus*) and the Andaman giant gecko (*Gekko verreauxii*). From the COI sequences of the Andaman day gecko, we detected 25 haplotypes across different island populations with a haplotype network typical of a large population with consistent gene flow between sampled islands, except the Little Andaman Island. We detected high levels of allelic diversity in microsatellite markers (43-12). A PCA with microsatellites revealed only one cluster, indicating lack of population differentiation. Softwares STRUCTURE and Delta K predicted one cluster, with weak signature of presence of another cluster, grouping the southern versus northern populations. This anomaly, when compared to structured genetic diversity of other endemic geckos on the Andaman Islands, suggests a possible role of human mediated dispersal of the Andaman day gecko, especially through trade of coconut and banana saplings. Using Approximate Bayesian Computation (ABC) models, we are testing three possible scenarios (a) a single stable population (b) an expanding population (c) population split and recent admixture, to understand factors which have led to the observed population structure.



**MORPHOLOGICALLY DISKINKT: COMPARING ISLAND POPULATIONS OF SKINKS IN THE COMOROS ARCHIPELAGO**

KATHLEEN C. WEBSTER

Ludwig-Maximilians-Universität, Munich, Germany  
Zoologische Staatssammlung München, Munich, Germany

OLIVER HAWLITSCHKE

Zoologische Staatssammlung München, Munich, Germany

MARK D. SCHERZ

Zoologische Staatssammlung München, Munich, Germany

FRANK GLAW

Zoologische Staatssammlung München, Munich, Germany

Studying island radiations of species helps us better understand the ecological and evolutionary forces behind diversification following island colonization. This study focuses on a radiation of fossorial skinks of the genus *Flexiseps*, highlighting specifically two species: *Flexiseps ardouini*, native to northern Madagascar and *F. johannae*, the only extant non-Malagasy species of *Flexiseps*, which is endemic to the Comoros Archipelago. A previous DNA barcoding study on Comoran squamate reptiles found *F. ardouini* nested within *F. johannae*, which was unexpected due to the allopatric distribution ranges and clear morphological distinction of the two species. Our study investigates whether further morphological and nuclear genetic data confirms this relationship. We found significant morphological differences among the four island populations of *F. johannae* and between the two species in morphometrics, pholidosis, and osteology, thus confirming the morphological distinction between *F. johannae* and *F. ardouini*. Many of the morphological differences between the species were size-related, with *F. johannae* presenting similar morphometric characteristics to *F. ardouini* just on a smaller scale. On the contrary, the two species shared identical haplotypes in both nuclear genetic markers studied. This incongruence between genetic data and morphology perhaps indicates either a historical human introduction or a recent natural dispersal of *F. johannae* from Madagascar followed by rapid morphological adaptation to the environments of the Comoros. The morphological variability of *F. johannae* is unique for skinks and suggests evidence of incipient speciation. *Flexiseps johannae* in the Comoros Archipelago is, therefore, an excellent new system to study the island biogeography of terrestrial reptiles.



# Workshop: Osteology in the XXI century



**THE OSTEOLOGY OF GECKOS: INTERESTING SKELETONS COME IN SMALL PACKAGES**

AARON M. BAUER

Villanova University, Villanova, Pennsylvania, USA

JUAN D. DAZA

Sam Houston State University, Huntsville, Texas, USA

Gekkotan lizards are the probable sister-group to all other squamates and have radiated into seven extant families and almost 1900 species. The group is characterized by highly kinetic skulls, large orbits, the loss of the supratemporal bar and parietal foramen, the ventral fusion of the subolfactory processes of the frontal, and posterior fusion of the Meckelian canal. Gecko skulls reflect the nocturnal ancestry of the group and demonstrate multiple episodes of miniaturization, as well as both paedomorphosis and peramorphosis. The vertebrae are usually amphicoelous and conservative in number, whereas the girdles, limbs and digits are highly variable and reflect locomotor specializations. Because of their small size and often extensive cartilaginous components, traditional osteological studies of the group were historically quite limited. The sequential development of radiography, clearing-and-staining and computed tomography (CT) has transformed gecko osteology since its modest beginnings. Although Paleogene geckos remain known chiefly from fragmentary remains, Cretaceous gecko morphology has been revolutionized by CT data from both sedimentary and amber fossils. These suggest that mid-Cretaceous geckos probably represent one or more lineages outside of the extant Gekkota. Among living geckos, the collection of near taxon-complete CTscans has augmented molecular phylogenies and provided the basis for the recognition and diagnosis of cryptic lineages (e.g., *Chatogekko*). Future directions for gecko osteology include enhanced study of the integration of phenotypic and molecular data, intraspecific skeletal variation – especially ontogenetic change, 3D-morphometric analysis of individual skeletal elements as well as entire skulls, and the application of finite element analysis to gecko morphology.

**THE HERPETOFAUNA OF A COASTAL TROPICAL FOREST OF MYANMAR,  
DURING THE MID-CRETACEOUS**

JUAN D. DAZA

Sam Houston State University, Huntsville Texas, United States

AARON M. BAUER

Villanova University, Villanova Pennsylvania, United States

Modern tropical forests harbor a high diversity of amphibians and reptiles. The abundant amber deposit from the Albian-Cenomanian boundary of Myanmar (99 Ma) has recently uncovered a large number of herpetological specimens embedded in amber. In recent years increased mining activities in Hukawng Valley, Northern Myanmar have yielded hundreds of fossils. A large sample of these fossils (70), together with published records, indicate the presence of anurans (?Alytoidea), salamanders, albanerpetontids, a large number of squamates including geckos, skinks, lacertoideans, iguanians, and anguimorphs (including snakes), and avian and non-avian dinosaurs. The study of these specimens using X-ray microtomography allows us to study in detail their osteology (and in many cases the soft anatomy, e.g. musculature and lepidosis) facilitating comparison with living groups. The amphibians and reptiles from the mid-Cretaceous show diagnostic features of modern groups, but in some cases they exhibit transitional morphologies (e.g. geckos with unfused frontal bones and two bones in the posterodorsal corner of the orbit). This is the first attempt to provide a thorough taxonomic assessment of a paleocommunity of amphibians and reptiles from a mid-Cretaceous locality.

**GLOBAL HERPETOLOGICAL OSTEOLOGY: A PRELIMINARY OVERVIEW ON THE EUROPEAN TAXA**

MASSIMO DELFINO

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy  
Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Barcelona, Spain

AARON M. BAUER

Department of Biology, Villanova University, Villanova, Pennsylvania, United States of America

MARCO CAMAITI

School of Biological Sciences, Monash University, Clayton, VIC, Australia

JAMES GARDNER

Royal Tyrrell Museum of Palaeontology, Drumheller, Alberta, Canada

GEORGIOS GEORGALIS

Department of Ecology, Comenius University in Bratislava, Bratislava, Slovakia

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

RAÚL O. GÓMEZ

CONICET-Departamento de Ciencias Geológicas and Departamento de Biodiversidad y Biología Experimental, Universidad de Buenos Aires, Buenos Aires, Argentina

ERWAN LORÉAL-MARON

UMR CNRS 6118 Géosciences, Université de Rennes 1, Rennes, France

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

LOREDANA MACALUSO

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

THALASSA MATTHEWS

Centre of Excellence for Palaeontology, Iziko Museums of South Africa, Cape Town, South Africa

MATTEO PILI

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

LUCA RACCA

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

SIMONE M. SEGHETTI

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

TORSTEN M. SCHEYER

University of Zurich, Palaeontological Institute and Museum, Zurich, Switzerland

LUKARDIS C.M. WENCKER

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

ANDREA VILLA

Bayerische Staatssammlung für Paläontologie und Geologie, München, Germany

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

Osteological information for extant amphibians and reptiles is scattered through numerous papers and books; this hinders a precise perception of what is known and what is not. In order to aggregate in a synthetic way the available published information and to determine which taxa and topics warrant further attention, we started to compile an online database that records, besides the species and where its osteology is presented, the following key data: anatomical region documented, intraspecific variation (e.g., individual, ontogenetic, sexual), nature of the information (e.g., description, figure or table, scoring for cladistic analysis, measurements for geometric morphometrics or finite element analysis), type of preparation/source of the information (e.g., dry skeleton, clearing and staining, tomography, X-rays, histological section), number of specimens analyzed, and if the osteological information is associated with the description of a new species. The first published work on this topic appears to be “De quadrupedibus digitatis [...]” issued posthumously in 1637 by Aldrovandi, which depicts, among others, a partial skeleton of *Salamandra salamandra* next to the whole animal, as well as a skull of *Caretta caretta*. The osteology for most of the currently recognized 80 amphibian and 144 European reptile species (see Sillero et al. 2014) has been at least partially described in about 300 publications so far: 72 amphibians and 118 reptiles. Despite this broad taxonomic coverage, few European species have detailed descriptions for their entire skeleton as most accounts are focused on the skull, with the exception of urodelans (mostly vertebrae) and turtles (mostly shell).

**IDENTIFICATION WITHOUT EXTERNAL CHARACTERS - DIGGING DEEPER  
IN THE SKULL MORPHOLOGY OF FOSSORIAL LEPTOTYPHLOPIDAE USING  
 $\mu$ CT**

MITALI JOSHI

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

CLAUDIA KOCH

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

Several taxonomic groups are composed of species which are difficult to determine based on external characters, which are frequently related to similar adaptations to the environment. The fossorial blindsnakes of the family Leptotyphlopidae are characterized by a superficial external morphology strongly influenced by their fossorial lifestyle favoring a worm like appearance. They commonly have small size, slender body, smooth shiny scales, roundish blunt head, eyes either reduced or placed under the scale. The cryptic fossorial lifestyle makes it very difficult to collect them and identification is frequently complicated due to high morphological convergence. In recent years, there is an increase in skeletal morphological studies to evaluate cranial and postcranial morphologies, often based on just one single specimen or involve only few species and provide different levels of detail. Few of these studies suspected dorsal skull bone morphology as a useful diagnostic character. However, until now it is hardly been used as a character for distinction at generic and species level. The aim of this study is to generate essential information for the majority of the currently recognized genera employing modern, non-invasive, high resolution and less time consuming micro-CT technique. The data generated can be further used to better understand the inter-generic and specific differences and similarities in this taxonomically complicated group.

**SEXUAL DIMORPHISM IN SHELL SHAPE AND SIZE OF EUROPEAN POND  
TURTLE IN SERBIA**

MARKO MARIČIĆ

University of Belgrade, Faculty of Biology, Institute of Zoology, Studentski trg  
16, 11000 Belgrade, Serbia

VUKAŠIN BJELICA

University of Belgrade, Faculty of Biology, Institute of Zoology, Studentski trg  
16, 11000 Belgrade, Serbia

ANA GOLUBOVIĆ

University of Belgrade, Faculty of Biology, Institute of Zoology, Studentski trg  
16, 11000 Belgrade, Serbia

Sexual dimorphism (SD) implies the difference between sexes in phenotypic characteristics. SD is a widely occurring phenomenon amongst animal kingdom, and it is well documented in turtles. We examined sexual size dimorphism (SSD) and sexual shape dimorphism (SShD) of shell, in three lowland populations of European pond turtle (*Emys orbicularis*) in Serbia. As measures of size we used linear measures (LM), centroid size (CS) and mass of 167 live specimens. In all of the examined populations, females tend to be the largest sex, concerning values for LM, CS and mass. We confirmed slight differences in female-biased SSD between the studied populations by calculating Lovich-Gibbons two-step ratio. Larger body size of females is assumed to be driven by the natural selection for fecundity. In aquatic environments, smaller body size of males can result in greater agility and, therefore, mating success. In the analysis on SShD of the plastron and carapace we used standard methods for 2D geometric morphometrics. Statistically significant differences in shape, of both plastron and carapace, are found between sexes. Plastron shape is shown to be more sexually dimorphic than carapace shape. Females tend to have narrower plastron with larger pectoral, abdominal and femoral scutes. Shape of the female carapace is more orbicular, unlike the male carapace that is flattened and trapezoid-like. As they grew larger, the carapace of males gravitates towards a more female-like shape.



**IDENTIFYING FOSSIL FROG ASSEMBLAGES FROM SOUTHERN AFRICA  
BASED ON A REVIEW OF THE ILIA OF EXTANT TAXA**

THALASSA MATTHEWS

Centre of Excellence for Palaeontology, Iziko Museums of South Africa, Cape Town, South Africa,

RACHEL KEEFFE

Department of Biology, University of Florida, Gainesville, Florida, United States of America

DAVID C. BLACKBURN

Florida Museum of Natural History, University of Florida, Gainesville, Florida, United States of America

Frog bones have been recovered from South African archaeological and palaeontological sites dating to the early Pliocene, Pleistocene and Holocene. These fossils generally remain unanalyzed as the result of a lack of comparative material to enable identification, despite their huge potential to provide valuable palaeo-climatic and environmental data. Iliia frequently make up a large portion of the anuran bones recovered from fossil sites world-wide, and have traditionally been used to identify fossil taxa. In order to address the lack of comparative information for southern Africa, a review was carried out on the ilia of frog taxa from Angola, Botswana, Malawi, Mozambique, Namibia, Lesotho, South Africa, Swaziland, Zambia, and Zimbabwe. Computed Tomography (CT scans) scans of all forty anuran genera – representing all thirteen families currently found in southern Africa - were carried out by the University of Florida's Nanoscale Research Facility (USA), and by Central Analytical Facilities at Stellenbosch University (South Africa). Four linear and two angle measurements were made on each ilium, and some 17 characters/features were assessed and described for each taxon. This review illustrates that ilia can enable clear differentiation on both a family and generic level, however, because there is so much variation in ilial features between taxa, the features used to identify a fossil will vary depending on the family and genus involved. The use of these review results is demonstrated by their application to some interesting fossil frog taxa from South Africa.

**COMPARATIVE CRANIAL OSTEOLOGY OF *Podarcis* LIZARDS (SQUAMATA: LACERTIDAE): EVOLUTIONARY AND PALAEOLOGICAL IMPLICATIONS**

TOMASZ SKAWIŃSKI

Department of Evolutionary Biology and Conservation of Vertebrates, University of Wrocław, Wrocław, Poland

EDYTA TURNIAK

Department of Evolutionary Biology and Conservation of Vertebrates, University of Wrocław, Wrocław, Poland

BARTOSZ BORCZYK

Department of Evolutionary Biology and Conservation of Vertebrates, University of Wrocław, Wrocław, Poland

The skull has played a crucial role in studying reptile evolution, systematics and palaeontology. In recent years, our knowledge about the cranial osteology of many European lacertids has improved significantly. Despite that, the data on the skull osteology are still very scarce for many lizards. One example is *Podarcis* – the most species-rich genus of European lacertids. In an attempt to fill part of this gap, we described in detail the skull morphology of four species of *Podarcis*: *P. muralis*, *P. cretensis*, *P. erhardii* and *P. siculus*. We created 3D models of studied specimens using photogrammetry before disarticulation. This allowed us to describe the characters unobservable in complete skulls without losing information recorded in fully articulated specimens. Superficially, *Podarcis* lizards are relatively similar to one another in their skull morphology. However, numerous qualitative and quantitative traits enable distinguishing the studied species. These include the characters such as the degree of development of the median crest on the ventral surface of the parietal, the extent of the parietal fossa (or lack thereof), morphology of the occipital condyle or the height of the ascending process of the supraoccipital. This knowledge may be useful in reconstructing the evolutionary history of *Podarcis* lizards and correctly assigning fossils, which are usually regarded as indeterminate. Additionally, we propose that even specimens of unknown geographical origin – though not useful in intraspecific studies – may have value as comparative specimens in palaeontology and are thus important in osteological collections.

**NEW DATA ON THE MIDDLE JURASSIC LIZARDS FROM GREAT BRITAIN**

MATEUSZ TAŁANDA

Department of Palaeobiology and Evolution, Faculty of Biology, University of Warsaw, Warsaw, Poland  
Affiliation, City, Nation (Centered - Times New Roman 9 – Exactly line spacing 12pt)

SUSAN EVANS

Department of Cell and Developmental Biology, University College London, London, United Kingdom

ROGER BENSON

Department of Earth Sciences, University of Oxford, Oxford, United Kingdom

VINCENT FERNANDEZ

Imaging and Analysis Centre, The Natural History Museum, London, United Kingdom

Molecular and palaeontological estimates suggest that the lizard crown group originated by the Middle Jurassic. Unfortunately, squamates are unknown from the Early Jurassic and, with the exception of two poorly preserved Chinese specimens, available material from the Middle Jurassic is mostly fragmentary and disarticulated. Incompleteness of this early fossil record hampers accurate polarization of characters during phylogenetic analyses, and limits our knowledge of early squamate diversification. Here we present a new, near-complete fossil squamate from the Bathonian (Middle Jurassic) of Skye (Kilmaluag Formation, Scotland). It has a snout-vent length of ca. 50 mm, and is the first articulated lizard skeleton from the Bathonian. The specimen was scanned using the European Synchrotron Radiation Facility in Grenoble, allowing high-resolution segmentation and digital dissection of individual bones from the skeleton. The frontal has Y-shaped groove dorsally, small parietal tabs on both sides and no trace of a postfrontal facet. Together with the chisel-like teeth, this indicates attribution to *Bellairsia gracilis*, which was previously known only from isolated frontals and jaw elements from Kirtlington (Forest Marble Series, England). The new skeleton confirms taxonomic attribution of isolated, disarticulated specimens from Kirtlington. *B. gracilis* displays a surprising mixture of advanced and primitive traits. The vertebrae are amphicoelous with a relatively wide notochordal canal. However, the postfrontals are miniaturized and the postorbital arch may have been reduced. Preliminary phylogenetic analyses using only morphological characters put *Bellairsia gracilis* as a sister taxon to “scleroglossans”. When the analysis is run using a backbone molecular constraint tree, in which Gekkota are the sister group to other squamates, then *Bellairsia* drops on to the squamate stem. This suggests that squamates had already undergone an early episode of diversification by the Middle Jurassic, which probably predates the origin of the crown.

**COMPARATIVE SKULL OSTEOLOGY AND PHYLOGENY OF THE ITALIAN VIPERS**

SIMONE MATTEO SEGHETTI

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

ANDREA VILLA

Bayerische Staatssammlung für Paläontologie und Geologie, München, Germany

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

EMANUEL TSCHOPP

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

Division of Paleontology, American Museum of Natural History, New York City, NY, USA

Universidade Nova de Lisboa, Faculdade de Ciências e Tecnologia, Caparica, Portugal

Museu da Lourinhã, Lourinhã, Portugal

FEDERICO BERNARDINI

Centro Fermi, Museo Storico della Fisica e Centro di Studi e Ricerche "Enrico Fermi", Roma, Italy.

Multidisciplinary Laboratory, the "Abdus Salam" International Centre for Theoretical Physics, Trieste, Italy

MICHELE MENEGON

Division of Biology & Conservation Ecology, Manchester Metropolitan University, UK

PAMS Foundation, Arusha, Tanzania

MASSIMO DELFINO

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Barcelona, Spain.

We studied the skulls of the five Italian species of *Vipera* to estimate intraspecific variability, detect diagnostic characters and perform a specimen-level phylogenetic analysis. The sample includes dry-prepared, disarticulated skulls and CT scans of five *Vipera aspis*; five *V. berus*; two *V. ammodytes*; two *V. ursinii*; and four *V. walser*. *V. aspis* displays a unique morphology in the premaxilla (lobe-like nasal process). *V. ammodytes* has unique morphologies in the premaxilla (rounded nasal process, wavy and spurred palatine process), in the palatine (up-turned anterior process), and in the pterygoid (forked anterior tip). Diagnostic characters for *V. ursinii* are the short maxilla, the short palatine with a non-forked posterior articular facet, the rounded anterior tip of the ectopterygoid, the long and slim splenial, and the supraoccipital and occipital crests that do not reach the parietal. *Vipera walser* is unique in having the occipital crest closer to the supraoccipital one. No single diagnostic feature could be identified in *V. berus*, but the species is recognizable by a combination of characters: a short postorbital articular facet and the trapezoidal premaxilla (different from *V. aspis* and *V. ammodytes*, but similar to *V. ursinii* and *V. walser*); a supraoccipital crest that is close to the parietal (as in *V. walser*, but different from *V. ursinii*); and an occipital crest that is distant from the supraoccipital crest (different from *V. walser*). The result of the morphological phylogenetic analysis is broadly comparable with that obtained on a molecular basis, suggesting that skull features have a reliable phylogenetic signal.

**THE GENUS *Adolfus* STERNFELD, 1912**

PHILIPP WAGNER  
Allwetterzoo Münster, Germany

The lacertid genus *Adolfus* Sternfeld, 1912 is endemic to Africa and included only three species until recently. The species of the genus show a high ecological variability, with *A. africanus* as a typical lowland rainforest species to *A. alleni* as a species restricted to alpine habitats. However, new research using an integrative approach of several methods has shown, that the genus is more diverse than previously thought. New species have been described and the results also show an intraspecific diversity in skull anatomy. Here, the results of several publications are summarized and future steps will be outlined.

**DIFFICULTIES IN USING CONTINUOUS CHARACTERS IN SPECIMEN-LEVEL  
OSTEOLOGICAL PHYLOGENETIC ANALYSES OF LACERTID LIZARDS**

LUKARDIS C. M. WENCKER

Dipartimento di Scienze della Terra, Università di Torino, Turin, Italy

EMANUEL TSCHOPP

American Museum of Natural History, Division of Paleontology, New York, USA

Dipartimento di Scienze della Terra, Università di Torino, Turin, Italy

Museu da Lourinhã, Lourinhã, Portugal

MASSIMO DELFINO

Dipartimento di Scienze della Terra, Università di Torino, Turin, Italy

Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Barcelona, Spain

Morphological phylogenetic analyses are mostly based on discrete characters or continuous characters that have been coded as discrete ones. However, the discretisation of a continuous character leads to a certain loss of information. Even though methods for analysing continuous character states have been developed, relatively little effort to estimate the contribution of those characters to the tree accuracy has been made until recently. In this study, we carried out specimen-level phylogenetic analyses of lacertid lizards with maximum parsimony. We sampled 34 ingroup and 4 outgroup species with up to 10 specimens scored per species. Our character matrix contained 250 characters describing osteological features only. 33 of the characters were continuous. We compared the tree topologies when treating all 250 characters as discrete, and when analysing the 33 continuous characters as such together with the 217 discrete ones. Tree accuracy was evaluated counting the number of specimens of a known biological species forming a clade. The overall tree resolution and recognition of species as clades seemed to be better for the combination of continuous and discrete characters than for discrete characters only. In addition, our results show that an increase in specimen sampling correlates with increasing tree accuracy. This is possibly due to better coverage of intraspecific variation which makes the individual variation carry less weight. However, phylogenetic relationships on higher taxonomic levels remained questionable when using continuous characters indicating that further modification is needed to have the complete analysis benefit from the extra bit of information gained from continuous characters.



Workshop: Developing a response plan to  
*Batrachochytrium salamandrivorans*



**BENEFICIAL AMPHIBIAN THERMAL BEHAVIOUR REMAINS CONSTRAINED BY THE ENVIRONMENT IN THE FACE OF PATHOGENIC INVASION**

WOUTER BEUKEMA

Wildlife Health Ghent, Dept. Pathology, Bacteriology and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

ALEXANDRA LAKING

Wildlife Health Ghent, Dept. Pathology, Bacteriology and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

SARAH VAN PRAET

Wildlife Health Ghent, Dept. Pathology, Bacteriology and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

JESSE ERENS

Wildlife Health Ghent, Dept. Pathology, Bacteriology and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

KRIS VERHEYEN

Forest & Nature Lab, Dept. Forest and Water Management, Ghent University, Geraardsbergsesteenweg 267, 9090 Gontrode, Belgium

FRANK PASMANS

Wildlife Health Ghent, Dept. Pathology, Bacteriology and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

AN MARTEL

Wildlife Health Ghent, Dept. Pathology, Bacteriology and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

When confronted with disease, amphibians profit from a rich line of defence that includes bioactive secretions, pathogen-inhibiting skin microbiota, and behavioural responses such as body temperature regulation. How the environment modulates expression and efficacy of these strategies however remains surprisingly unclear. Here, we focus on European emergence of the fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*) to identify drivers and constraints that shape the thermal dimension of amphibian disease ecology. We first map environmental context by assembling a primer on thermal ecology of our focal host *Salamandra salamandra*, while taking that of syntopic reservoir species into account as well. Three years of field surveys, thermal preference experiments and a mesocosm trial allow us to determine how body temperatures fluctuate within this assemblage in respect to its environment. We then narrow our focus to *S. salamandra* host thermal behaviour, which we experimentally record before and after exposure to *Bsal*. We find that thermal behaviour inhibits *Bsal* infection – yet, this cannot be expressed under natural conditions. Instead, wild *S. salamandra* thermoconform, with body temperatures remaining at *Bsal*-favourable conditions of 10-20°C during most of the year. We use these data to inform a mechanistic model that predicts microhabitat invasibility to *Bsal* throughout Europe. While building on a local example, we emphasize that diversity of amphibian body-environment interactions is limited, for instance illustrated by strong ecological niche conservatism among salamanders worldwide. Amphibian thermal behaviour and its function as buffer to disease, but also climate change, may therefore be compromised among many more species than hitherto assumed.

## EMERGING INFECTIOUS DISEASES OF AMPHIBIANS IN HUNGARY

DÁVID HERCZEG

Lendület Evolutionary Ecology Research Group, Plant Protection Institute, Centre for  
Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

JAIME BOSCH

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

JUDIT VÖRÖS

Department of Zoology, Hungarian Natural History Museum, Budapest, Hungary

Amphibian declines are emblematic of the current extinction event on Earth. Beyond many factors diseases are identified as main stressors of global decline. Fungal pathogens such as *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* are responsible for chytridiomycosis and viruses of the genus *Ranavirus* (Rv) also present a considerable disease risk for amphibians. Our survey primarily focused on the distribution and host range of Bd and Rv in Hungary, a country with high amphibian diversity due to different climatic and zoogeographical influences. Moreover, to explore the historical distribution of Bd in Hungary field surveys were complemented with available archived samples of *Bombina* spp. (N=127) from museum collections between 1936-2005. We sampled 16 taxa (N=1233) in the field for Bd and 7 taxa were infected with the overall prevalence of 7.46% (95% CI:6.05-9.07). All of the archived *Bombina* spp. samples were Bd negative. Eight taxa (N=137) were sampled during the Rv surveillance and 7 taxa were infected with the virus. The overall prevalence was 41.6% (95% CI:0.34-0.50). The most frequently infected species with Bd were *Pelophylax ridibundus* (12.80%; 95% CI:8.10-18.91) and *Bombina variegata* (12.70%; 95% CI:9.92-15.92). In contrast, Rv infection frequency were the highest in *Bufo bufo* (50%; 95% CI:0.24-0.76) and *Salamandra salamandra* (50%; 95% CI:0.30-0.69) samples. We have to note that all sampled individuals apparently didn't display any clinical sign of chytridiomycosis or ranavirosis, but changes in the climate might alter the diffusion of pathogens and make their spread less predictable, thus areas not yet affected by epidemics require particular attention and constant monitoring.

**RECENT FINDINGS OF THE AMPHIBIAN KILLER FUNGUS *Batrachochytrium salamandrivorans*, THE SPREADING GOES ON**

DAVID LASTRA GONZÁLEZ

<sup>1</sup>Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Czech Republic.

VOJTECH BALÁŽ

Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

MILIČ SOLSKÝ

<sup>1</sup>Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Czech Republic.

BARBORA THUMSOVÁ

<sup>1</sup>Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Czech Republic.

KRZYSZTOF KOLENDA

Department of Evolutionary Biology and Conservation of Vertebrates, University of Wrocław, Poland.

ANNA NAJBAR

Department of Evolutionary Biology and Conservation of Vertebrates, University of Wrocław, Poland.

MARIA OGIELSKA

Department of Evolutionary Biology and Conservation of Vertebrates, University of Wrocław, Poland.

MATEJ KAUTMANN

Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

MONIKA BALGOVÁ

Institute of Biology and Ecology, Pavol Jozef Šafárik University in Košice, Slovakia.

PETR CHAJMA

<sup>1</sup>Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Czech Republic.

JIRÍ VOJAR

Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Czech Republic.

The recently discovered pathogen *Batrachochytrium salamandrivorans* (Bsal) has severe impacts mainly on European salamanders and newts. This emerging chytrid fungus, which provokes lethal effects over the skin of infected caudates, likely arrived in Europe by pet trade from South-east Asia, where its presence has been confirmed with no related mortalities. The occurrence of Bsal in Europe has already been confirmed in the Netherlands, Belgium and Germany in the wild and in the UK, Germany and Spain in captive animals. In our study, carried out in six European countries and analyzing more than 1100 samples, we have found the presence of Bsal in the wild in Spain for the first time. These results were found in five *Lissotriton helveticus*, a species previously thought to be Bsal-resistant. Most Bsal positives were in drinking troughs in the Cantabrian Mountain Range, a mountainous area in northern Spain. Together with the control of amphibian trade implementing the recent decision of European Union (EU) no. 2018/320 for intra-Union trade in salamanders and their introduction into EU, the knowledge of the current geographic range of pathogens is essential for establishing effective measures in affected areas and preventing introduction in Bsal-free regions.

**GERMANY, THE HOTSPOT OF BSAL EMERGENCE**

STEFAN LÖTTERS

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

MICHAEL VEITH

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

LUTZ DALBECK

Biologische Station im Kreis Düren e.V., Zerkaller Straße 5, 52385 Nideggen, Germany

HEIDRUN DÜSSEL-SIEBERT

Biologische Station im Kreis Düren e.V., Zerkaller Straße 5, 52385 Nideggen, Germany

STEPHAN FELDMEIERS

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

MAIKE GUSCHAL

Biologische Station StädteRegion Aachen, Zweifaller Straße 162, 52224 Stolberg/Rheinland, Germany

KAI KIRST

Biologische Station StädteRegion Aachen, Zweifaller Straße 162, 52224 Stolberg/Rheinland, Germany

DAGMAR OHLHOFF

Biologische Station im Kreis Düren e.V., Zerkaller Straße 5, 52385 Nideggen, Germany

MARTIN SCHLÜPMANN

Biologische Station Westliches Ruhrgebiet, Ripshorster Straße 306, 46117 Oberhausen, Germany

KATHLEEN PREIßLER

Universität Leipzig, Institut für Biologie, Molekulare Evolution und Systematik der Tiere, Talstraße 33, 04103 Leipzig, Germany

JOANA SABINO-PINTO

Technische Universität Braunschweig, Zoologisches Institut, Mendelssohnstraße 4, 38106 Braunschweig, Germany

VANESSA SCHULZ

Universität Leipzig, Institut für Biologie, Molekulare Evolution und Systematik der Tiere, Talstraße 33, 04103 Leipzig, Germany

SEBASTIAN STEINFARTZ

Universität Leipzig, Institut für Biologie, Molekulare Evolution und Systematik der Tiere, Talstraße 33, 04103 Leipzig, Germany

MIGUEL VENCES

Technische Universität Braunschweig, Zoologisches Institut, Mendelssohnstraße 4, 38106 Braunschweig, Germany

NORMAN WAGNER

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

JOSEF WEGGE

Biologische Station StädteRegion Aachen, Zweifaller Straße 162, 52224 Stolberg/Rheinland, Germany

Since its discovery in the Netherlands 10 years ago, numerous new records of *Batrachochytrium salamandrivorans* (*Bsal*) suggest that this invasive pathogen is extending its exotic range in Europe rapidly. Germany is the ‘hotspot’ of *Bsal* emergence. Of the nearly 50 European sites known by early 2019, almost 40 are located in Germany affecting five urodelan species: *Salamandra salamandra* >20 populations, *Ichthyosaura alpestris* >10 populations, *Lissotriton vulgaris* and *Lissotriton helveticus* 5 and 4 populations, respectively, and *Triturus cristatus* 2 populations (double-counts because of co-occurrence of two or more species at the same site). Especially, *S. salamandra* suffers from *Bsal* infection, as verified by dramatic population declines and sometimes even mass mortality events. In one such case co-infection with *Batrachochytrium dendrobatidis* was also detected. Records in Germany are known from two regions: (1) The Eifel Mountains in the West close to Dutch and Belgian outbreaks; in this region, *Bsal* presence could be dated back to 2004; (2) the Ruhr, where *Bsal* was first detected in 2017 and is currently spreading at alarming rates. At several sites in the Eifel Mountains, historically known to harbor *S. salamandra* and newts, nowadays only newts can be found. Some of these are *Bsal*-positive, suggesting silent *Bsal*-induced salamander declines in the past. In other *Bsal* sites both in the Eifel Mountains and the Ruhr, we still find fire salamanders at low densities. Standardized larval monitoring in the southern Eifel Mountains revealed recent breakdowns of fire salamander populations, thus identifying part of the *Bsal* expansion front. Our data give new insight into *Bsal* invasion dynamics. This demonstrate that monitoring is an essential key to understand the threat posed by this pathogen to Europe’s salamanders.

**SETTING THE SCENE: THE BSAL THREAT TO EUROPEAN URODELES**

FRANK PASMANS

Wildlife Health Ghent, Ghent University, Merelbeke, Belgium

AN MARTEL

Wildlife Health Ghent, Ghent University, Merelbeke, Belgium

The chytrid fungus *Batrachochytrium salamandrivorans* (Bsal) is posing an unprecedented infectious threat to European urodele diversity. Current uncertainties translate to taking drastic mitigation actions (removing hosts, disinfection, erecting barriers etc). Here, we will present an overview of recent insights in the host-pathogen-environment interaction that help understand disease dynamics and assist in designing better informed and more targeted mitigation. Although the link between conducting basic research and actual real world conservation may not be obvious, we will provide examples of how fundamental insights are necessary to limit the impact of amphibian diseases. For example, understanding determinants of host susceptibility allows prioritization, vaccination and probiotic mitigation attempts, and the identification of susceptibility markers, which can be used for marker-assisted selective breeding. Understanding pathogen virulence may contribute to improved diagnostics, treatment and even prevention (vaccination). Elucidating the contribution of the environmental context may allow predicting impact, identifying refuges and guiding habitat management. Reducing uncertainty should help fine-tuning the early warning system and the disease response plans.

**SAVING THE FIRE SALAMANDER: EX SITU BREEDING OF INSURANCE POPULATIONS IN THE FACE OF AN EXPANDING PATHOGEN**

KATHLEEN PREISLER

University Leipzig, Institute of Biology, Leipzig, Germany

MIGUEL VENCES

Technische Universität Braunschweig, Zoological Institute, Braunschweig, Germany

SEBASTIAN STEINFARTZ

University Leipzig, Institute of Biology, Leipzig, Germany

In the wake of rapidly declining global biodiversity, conservation management has to focus on the preservation of populations to maintain and maximize the genetic diversity and the evolutionary potential of a species. Globally, biodiversity is threatened by climate change, pollution, and habitat destruction as well as by emerging infectious diseases. Currently, *Batrachochytrium salamandrivorans* (Bsal), an introduced pathogenic chytrid fungus is expanding through Central Europe and has been severely affecting populations of the fire salamander (*Salamandra salamandra*), leading to rapid declines and regional extinctions. In the face of a threat bringing this species potentially to the brink of extinction, *ex situ* management of fire salamander populations has been suggested as one of the most promising approaches to conserve the genetic diversity as a last resort. We conducted a study aiming to identify management units of the fire salamander in Germany, the species' main area of distribution, where Bsal is rapidly spreading. We sampled ~60 populations of *S. salamandra* across Germany and determined their genetic composition by microsatellite analysis, RAD sequencing and mitochondrial sequence analysis. The identified genetic units represent the most important populations worth being protected and the candidates for an *ex situ* project in Germany to save the genetic diversity and adaptations present.

***Batrachochytrium salamandrivorans* IN THE NETHERLANDS: PAST, PRESENT AND FUTURE PERSPECTIVES**

ANNEMARIEKE SPITZEN – VAN DER SLUIJS

Reptile, Amphibian and Fish Conservation the Netherlands, Nijmegen, the Netherlands

MAARTEN GILBERT

Reptile, Amphibian and Fish Conservation the Netherlands, Nijmegen, the Netherlands

The chytrid fungus *Batrachochytrium salamandrivorans* [Bsal] was first detected in a population of fire salamanders (*Salamandra salamandra*) in the Netherlands. Since its detection over 10 years ago, research on this topic has accelerated and has led to novel insights. However, if this knowledge is not translated, or is not accepted by policy makers, conservation will not be successful. This presentation will provide an overview of our experiences in the Netherlands. We will present the set of actions that was initiated immediately after the discovery of Bsal and the subsequent decline of fire salamanders in this country. Moreover, we will discuss the importance of translating scientific findings to management layers and we will discuss the lessons we have learnt to improve future options for the detection and management of new outbreaks.

**AMPHIBIAN POPULATION MANAGEMENT IN SOUTHERN EUROPE TO  
CONTAIN AND ELIMINATE A LOCAL OUTBREAK OF *Batrachochytrium  
salamandrivorans* (BSAL)**

MIREIA VILA-ESCALÉ

Oficina Tècnica de Parcs Naturals. Diputació de Barcelona, Barcelona, Spain

DANIEL FERNÁNDEZ-GIBERTEAU

Grup de recerca de l'Escola de Natura de Paret del Vallès (GREN), Paret del Vallès, Spain

SALVADOR CARRANZA

Institute of Evolutionary Biology (IBE-CSIC-UPF), Barcelona, Spain

ALBERT FERRAN

Oficina Tècnica de Parcs Naturals. Diputació de Barcelona, Barcelona, Spain

PEP PANNON

Oficina Tècnica de Parcs Naturals. Diputació de Barcelona, Barcelona, Spain

MARIONA PICART

Oficina Tècnica de Parcs Naturals. Diputació de Barcelona, Barcelona, Spain

LAIA PÉREZ-SORRIBES

Grup de recerca de l'Escola de Natura de Paret del Vallès (GREN), Paret del Vallès, Spain

CAROLINA MOLINA

Grup de recerca de l'Escola de Natura de Paret del Vallès (GREN), Paret del Vallès, Spain

AÏDA TARRAGO

Servei de Fauna i Flora. Generalitat de Catalunya, Barcelona, Spain

STEFANO CANESSA

Faculty of Veterinary Medicine. Department of pathology, bacteriology and poultry diseases. Ghent University, Ghent, Belgium

AN MARTEL

Faculty of Veterinary Medicine. Department of pathology, bacteriology and poultry diseases. Ghent University, Ghent, Belgium

FRANK PASMANS

Faculty of Veterinary Medicine. Department of pathology, bacteriology and poultry diseases. Ghent University, Ghent, Belgium

We report an outbreak of Bsal in a wild amphibian population of southern Europe. The infection was detected in March 2018, in a small reservoir inside a natural protected area of Catalonia (northeastern Spain), resulting in high mortality of *Triturus marmoratus*. Analyses of the amphibian population detected Bsal, *B. dendrobatidis* (Bd) and Ranavirus. The early detection of the chytrid infection facilitated a quick intervention to try to eradicate the outbreak by isolating the infected area (“ground zero”), managing the affected amphibian population and monitoring the surrounding populations within the same valley. The infected area was fenced to prevent the access. The stream that fed the reservoir was diverted and a central pond was modified as an amphibians trap to remove as much animals from the population as possible. The traps and ground zero were checked twice a week and all amphibians were preserved and tested for emergent diseases. Temperature data loggers were placed for long-term monitoring. A strict biosecurity protocol has been in place and the workers involved have been fully trained. 1,323 individuals of 10 amphibian species were captured in 2018 and, in autumn-winter 2018-19, all sampled individuals tested negative. In spring 2019, new cases of Bsal infection were detected, affecting *T. marmoratus* and *Salamandra salamandra*. The infection has not extended beyond ground zero. Interestingly, Bd has not been detected since autumn 2018. This finding demonstrates that even quick and rigorous action is not sufficient to eradicate Bsal from natural populations and stresses the need for long-term disease management.



**DEVELOPING AN EARLY RESPONSE PLAN TO INVASION BY *Batrachochytrium salamandrivorans***

STEFANO CANESSA

Wildlife Health Ghent, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

CLAUDIO BOZZUTO

Wildlife Analysis GmbH, Zurich, Switzerland

FRANK PASMANS

Wildlife Health Ghent, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

AN MARTEL

Wildlife Health Ghent, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Six years have passed since *Batrachochytrium salamandrivorans* (Bsal) was identified as the driver of salamander extirpations in Northern Europe. Bsal has been detected in captive collections throughout the continent, so spillovers could occur anywhere at any time. The contrasting experiences in countries affected to date demonstrate the challenge of mitigating a Bsal outbreak, but also suggest some room for action if we can prepare in advance and react quickly.

Ecological theory and experimental data give a general idea of the effort required to contain and eradicate an outbreak. Our results suggest actively removing  $\geq 85\%$  of focal species and pathogen reservoir species has an approximate 50% chance of eradicating Bsal; free-living persistent zoospores and seasonal infection dynamics can further add to the intensity of management needed (up to 10% and 33% extra effort respectively, although these values are quite uncertain).

Beyond these scientific insights, non-biological aspects of mitigation can also be problematic. Even assuming invasion can be detected early enough, time will be extremely limited. We should (1) identify in advance who is responsible for making decisions, including permits and funding, (2) think practically and share knowledge about actions like building fences, removing hosts and manipulating habitats, (3) be prepared to deal with uncertainty upon detection (when and how did Bsal arrive? How widespread is it around the detection site?).

Overcoming these complications will be difficult in itself. However, Bsal invasions in European regions of high salamander diversity can have catastrophic effects, which we should try to avoid at all costs.



# Workshop: Reassessment of red list status of European amphibians

**REASSESSMENT OF RED LIST STATUS OF EUROPEAN AMPHIBIANS**

JELKA CRNOBRNJA-ISAILOVIĆ

Faculty of Sciences and Mathematics University of Niš, 18000 Niš & Institute for Biological Research “S. Stanković”  
University of Belgrade, 11000 Belgrade, Serbia

DAN COGALNICEANU

Faculty of Natural Sciences and Agricultural Sciences University Ovidius Constanta, Constanta, Romania

MATHIEU DENOËL

Biology of Behaviour Freshwater and Oceanic science unit of research (FOCUS), University of Liège, 4020 Liege, Belgique

GENTILE FRANCESCO FICETOLA

Department of Environmental Science and Policy – ESP, University of Milan, 20133 Milan, Italy

TONY GENT

Amphibian and Reptile Conservation, Bournemouth, Dorset BH1 4AP, United Kingdom

ANDREAS MALETZKY

Department of Ecology and Evolution, University of Salzburg, A-5020 Salzburg, Austria

RAOUL MANENTI

Department of Environmental Science and Policy – ESP, University of Milan, 20133 Milan, Italy

CLAUDE MIAUD

Biogéographie et Ecologie des Vertébrés, PSL Research University, CEFE UMR 5175, 34293 Montpellier, France

IÑIGO MARTINEZ-SOLANO

National Museum of Natural History, 28006 Madrid, Spain

KATJA POBOLJŠAJ

Center for Fauna and Flora Cartography, SI-2204 Miklavž na Dravskem polju, Slovenija

RIINU RANNAP

Department of Zoology, Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia

BENEDIKT SCHMIDT

Department of Evolutionary Biology and Environmental Studies, University of Zurich, 8057 Zurich, Switzerland

ULRICH SCHULTE

Faunistic Expertise Office - Dr. Ulrich Schulte, 33829 Borgholzhausen, Germany

KONSTANTINOS SOTIROPOULOS

Department of Biological Applications and Technology, University of Ioannina, GR-45110 Ioannina, Greece

MATTHIAS STÖCK

Leibniz-Institute of Freshwater Ecology and Inland Fisheries – IGB (Research cooperation Berlin), D-12587 Berlin,  
Germany

NAZAN ÜZÜM

Faculty of Arts and Sciences, Adnan Menderes University, 09010 Aydın, Turkey

JUDIT VÖRÖS

Department of Zoology, Hungarian Natural History Museum, H-1088 Budapest, Hungary

JOHN WILKINSON

Amphibian and Reptile Conservation, Bournemouth, Dorset BH1 4AP, United Kingdom

JENNIFER LUEDTKE

Manager of IUCN Red List Assessments - Global Wildlife Conservation & Global Coordinator - Amphibian Red List  
Authority - IUCN SSC Amphibian Specialist Group

The IUCN Amphibian Red List Assessment should be updated every 10 years and the last one was completed in 2009. In 2018 at the SEH meeting in Salzburg, a new regional Amphibian Red List Assessor Team has been established and their main goal is to prepare draft (re)assessments for amphibian species inhabiting Europe. The (re)assessments are grouped according to three main themes – reassessments of the species previously assigned as threatened (21), assessments of newly proclaimed and data deficient species (5 or 6), and reassessments of the species previously evaluated as Least Concerned and Near Threatened (66 overall). According to the procedure, the drafts of all (re)assessments should be sent to third parties for review. Therefore, the opportunity to present the draft (re)assessments to the wide SEH audience adds an extra value to this Red Listing process through collecting valuable comments that can improve the final product – an updated Red List status of European amphibians. Recently, there are numerous indications that some species previously assigned as not threatened (due to wide range and absence of any evidence on their population status) in fact are decreasing in number. This symposium will facilitate review of the new evidences and help in properly assigning conservation status to the 93 species of European amphibians.



# POSTER presentations

**POPULATION DENSITY AND DISTRIBUTION OF MARMARIS LYCIAN  
SALAMANDER**

EYUP BAŞKALE

Pamukkale University, Faculty of Arts and Science Department of Biology,, Denizli, Turkey

DOĞAN SÖZBİLEN

Pamukkale University, Acıpayam Vocational School, Pamukkale University, Denizli, Turkey

YAĞMUR ÖZYILMAZ

Pamukkale University, Faculty of Arts and Science Department of Biology,, Denizli, Turkey

Marmaris Lycian Salamanders (*Lyciasalamandra flavimembris*) is one of the important endemic urodelan species of Anatolia. It generally lives in rocky limestone outcrops, and is often found in marquis scrub or pine woodlands. It also categorized as an Endangered (EN) species because of restricted distribution range, habitat loss caused by forest fires, and over-collection for scientific purposes. To re-evolution of the population status of Marmaris Lycian Salamanders, we conducted field studies in their known and possible distribution localities in Muğla Province. We found the new localities of the species that are represented by at least 13 populations in the province of Muğla Province. Individuals prefer the areas that are more north facing, with less sunlight, moist, and covered with vegetation where they can hide on the ground. The elevation ranged from 0 to 890 m and the mean temperatures range was 8 to 18 °C during the peak of active period. We also estimated population density that are varied 1-14 individuals/hectare among populations. Conservation action was started in order to protect and to long term survival of Marmaris Lycian Salamanders' populations.

**SNAKES OF THE STATE OF PARAÍBA, BRAZIL: CURRENT KNOWLEDGE**

RAFAELA CÂNDIDO DE FRANÇA

Programa de Pós-graduação em Ecologia e Conservação da Biodiversidade, Universidade Estadual de Santa Cruz, Brazil

DENNIS RÖDDER

Herpetology Section, Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

MIRCO SOLÉ

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Brazil

FREDERICO GUSTAVO RODRIGUES FRANÇA

Departamento de Engenharia e Meio Ambiente, Centro de Ciências Aplicadas e Educação, Universidade Federal da Paraíba, Brazil

South America harbours one of the richest herpetofaunas in the world, however, knowledge about the snakes of the biomes present in the Northeast Region of Brazil is still sparse and even basic information on natural history on most species is scarce and nonexistent. Our work aims to present an inventory of snake species registered in different phytophysiognomies of the state of Paraíba, addressing diversity, natural history and geographical distribution. Paraíba is located in the Northeast of Brazil and has an area of approximately 56 km<sup>2</sup>. Two Biomes are located within the state, the Atlantic Forest, covering the coastal zone, and the Caatinga Biome, located in most of the interior of the state. We revised data from 2.625 specimens from the collection of the Universidade Federal da Paraíba, gathered data during fieldwork and complemented with additional literature data. The list of species presented here corresponds to a total of 61 species distributed in 38 genera and 6 families. The Atlantic Forest of Paraíba has about 56 species of snakes, 42% of the total species registered for the whole Biome and the Caatinga of Paraíba has 22 species, which corresponds to 21% of the total recorded for the whole Biome. Although many areas of these two Biomes have already been lost due to deforestation, the state of Paraíba still shows a high diversity of snake species, being a priority region for the knowledge and conservation of these Biomes.



**NEW DATA ON *Hemidactylus arnoldi* IN THE HORN OF AFRICA**

ANNAMARIA NISTRI

Museo di Storia Naturale, Università di Firenze, Via Romana 17, Firenze, Italy

In 1933 the British lieutenant R.H.R. Taylor collected two geckoes in a locality of the Guban region, a dry area of the north-western Somalia, near to the border with Djibouti. The specimens, preserved in the British Museum of Natural History, were identified as belonging to a new species, described by B. Lanza in 1978 and named *Hemidactylus arnoldi*.

The gecko was no longer found but several authors supposed that the species could be reasonably present also in adjacent areas of Somaliland, Ethiopia and Djibouti. During two survey carried out in 2016 and 2019 in the Republic of Djibouti by the Natural History Museum and the Department of Biology of the University of Florence (Italy) two new specimens were collected. Although the whole country is characterized by a particularly dry environment, *H. arnoldi* was found in one of the less arid zones of Djibouti (near Dittilou's village, not so far from the Day Forest National Park, central Djibouti), at an elevation of about 700 m a.s.l. Measurements and scale counts are compared with those of Lanza's description. These findings represent the northernmost locality for *H. arnoldi* and a new species for Djibouti's herpetofauna.

**DIVERSITY BETWEEN FOUR REGIONS – HERPETOFAUNA IN THE TIRAS  
MOUNTAINS, NAMIBIA**

IRIS MADGE PIMENTEL

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

DENNIS RÖDDER

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

Transition zones between biogeographic units are often characterized by high species diversity. The availability of distinct habitat types within these transition zones allows for the coexistence of components from different species communities. Particularly lizard diversity in arid areas correlates with habitat heterogeneity, rendering transition zones promising for herpetological inventories. In the present study, herpetological diversity in the Tiras Mountains, a transition zone between four ecoregions in the southern Namibian Escarpment, is explored. We address the question, if the lizard community in this area is comprised of typical components of all adjacent ecoregions – the Namib desert, Namibian savanna woodlands, Succulent Karoo and Nama Karoo. By comparing the lizard community in the Tiras Mountains to those of national protected areas in the four ecoregions, we showed that the study area indeed harbors a mixed lizard community. There is no particular similarity to any of the ecoregions but greatest resemblance is observed for a Nama Karoo-savanna transition zone. Characteristic species from different ecoregions coexist in the study area. In this context, spatial partitioning appears to be an important mechanism for coexistence of closely related species. Although the Tiras Mountains did not show an exceptional diversity, this area supports an interesting species assemblage and even harbors regionally endemic lizards. It is a promising study area for ecological studies on reptile communities.

**NOTES ON AN ALPINE POPULATION OF THE ASP VIPER (*Vipera aspis*) IN NORTHWESTERN ITALY**

SONIA ACIERNO

Department of Neuroscience, University of Torino, Torino, Italy

MARCO SASSOÈ-POGNETTO

Department of Neuroscience, University of Torino, Torino, Italy

We present preliminary data of an ongoing field study on a population of the asp viper (*Vipera aspis*) in montane-subalpine habitat in northwestern Italy. The field of study is located between 1420 and 1580 m asl on a south facing valley slope and is characterized by open pastures surrounded by woodland. Since 2015, vipers have been surveyed systematically, largely during spring and autumn. Individual snakes were captured and photographed (head and body) for further recognition, and released at the exact site after collecting data on body size, microhabitat and activity.

Until present, a total of 87 individuals have been recorded (40 males, 30 females and 17 juveniles), with 37 vipers recaptured at least once. The highest number of recaptures was 6 (an adult male), although the majority of individuals (24) were recaptured only once or twice. Among the recaptured vipers, some individuals (7 males, 2 females) exhibited a remarkable nomadic behavior, covering on average a linear distance of  $119.1 \pm 43.3$  m (range: 65-173 m) between the first and last capture sites. Notably, a young male (total length 55 cm) moved 135 m between two sites in 9 days.

By using this approach, we plan to investigate population characteristics and ecology over time and obtain data on growth trajectory and dispersal ability of individual snakes. Of particular relevance will be the assessment of the impact of long-term habitat changes, largely caused by progressive abandon of pastures traditional management and subsequent reforestation, on population dynamics.

**PREDICTIVE SPECIES DISTRIBUTION MODELLING AS TOOL FOR STUDYING  
GEOGRAPHIC DISTRIBUTION OF THE RATTLESNAKE *Crotalus durissus* IN  
BRAZIL**

JESSICA VIVIANE AMORIM FERREIRA

Centro de Conservação e Manejo de Fauna da Caatinga, Universidade Federal do Vale do São Francisco, Petrolina, Brazil

MARLLA ALVES MATOS

Centro de Conservação e Manejo de Fauna da Caatinga, Universidade Federal do Vale do São Francisco, Petrolina, Brazil

LUCIANO MODESTO NASCIMENTO MENEZES

Centro de Conservação e Manejo de Fauna da Caatinga, Universidade Federal do Vale do São Francisco, Petrolina, Brazil

GEANE LIMEIRA DA SILVA

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

EUVALDO MARCIANO-JR

Centro de Conservação e Manejo de Fauna da Caatinga, Universidade Federal do Vale do São Francisco, Petrolina, Brazil

PATRICIA AVELLO NICOLA

Centro de Conservação e Manejo de Fauna da Caatinga, Universidade Federal do Vale do São Francisco, Petrolina, Brazil

MIRCO SOLÉ

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil and Herpetology Section,  
Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

Predictive species distribution modelling (SDM) is a widely used tool for conservation strategies planning of species in different contexts (rare, invasive or threatened species for example). Herein, we employed SDM in order to assess the current and potential distribution of the rattlesnake *Crotalus durissus* in Brazil. We generated distribution maps based on records available in the Specieslink database as well as from field data collected in areas of the herpetological monitoring program of the Integration Project of the São Francisco River (PISF, in Portuguese). We used Maxent to generate the environmental model. In total, 143 distribution records were recovered in Brazil, presenting a critical gap of records in the Caatinga Biome, with more than 75% of records related to the south and southeastern regions of Brazil. The resulting model showed an AUC = 0.962. This index evaluates the efficiency of the generated model and ranges from 0 (unreliable) to 1 (very reliable). We found that *C. durissus* presents a high potential of distribution in the Caatinga and transitional areas between Atlantic Forest and Cerrado Biomes in their central and southeastern portions.

**SPREAD OF *Trachemys scripta* SSP. (TESTUDINES, EMYDIDAE) IN SERBIA, WITH  
OVERVIEW OF POSSIBLE REPRODUCTION**

ALEKSANDAR UROŠEVIĆ

Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia

MILOŠ POPOVIĆ

Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, Niš, Serbia

MARKO MARIČIĆ

Scientific Research Society of Biology and Ecology Students "Josif Pančić", Novi Sad, Serbia

GORDAN POMORIŠAČ

Bird Study and Protection Society of Serbia, Novi Sad, Serbia

DRAGIŠA PETROVIĆ

Society of birds and nature lovers "Owls on alert", Čačak, Serbia

DAVID GRABOVAC

Association of environmentalists "Riparia", Subotica, Serbia

ALEKSANDRA SURLA

Scientific Research Society of Biology and Ecology Students "Josif Pančić", Novi Sad, Serbia

IVAN MEDENICA

Institute for nature conservation of Serbia, Niš, Serbia

STEFAN AVRAMOVIĆ

Institute of Zoology, Faculty of Biology, University of Belgrade, Belgrade, Serbia

ANA GOLUBOVIĆ

Institute of Zoology, Faculty of Biology, University of Belgrade, Belgrade, Serbia

The Red-eared slider (*Trachemis scripta elegans*) is among the world's most invasive species, and recently its related subspecies (*T. s. scripta* and *T. s. troostii*) were also being introduced in Europe. Herein, we present data on introduction of the Red-eared slider and its related subspecies in Serbia, gathered during more than ten years. The number of reports of these turtles across Serbia almost doubled since 2015. The introductions are concentrated around Belgrade and Novi Sad cities where large populations were detected, and all cases of possible reproduction: nest digging females and findings of hatchlings. The lakes, ponds and canals in the lowland habitats (less than 100 m a.s.l.) were more vulnerable to the introduction of alien turtles – more than 73% of found alien turtles were detected at low altitudes. The two other subspecies (*T. s. scripta* and *T. s. troostii*), which emerged in the pet trade as a "substitute" after the ban on import and trade of Red-eared sliders, were also recorded at the few localities. They can hybridize with the Red-eared slider (*T. s. elegans*) and possibly increase its invasive potential. The stricter enforcement of the existing legislature in Serbia is needed, together with raising public awareness of the problem and eradication of established populations. Building of turtle sanctuaries is also suggested as an ethical and ecologically safe alternative for disposal of unwanted pets.

## HERPETOLOGICAL SURVEYS IN CENTRAL ETHIOPIAN HIGHLANDS

MARCO A. L. ZUFFI

Natural History Museum Pisa, University of Pisa, Calci (Pisa), Italy

ELISABETTA PALAGI

Department of Biology - University of Pisa, Pisa, Italy

IVAN NORSCIA

Dipartimento di Scienze della Vita e Biologia dei Sistemi - Life Sciences and System Biology (DBIOS), Turin University, Turin, Italy

MARTA CASELLI

Dipartimento di Scienze della Vita e Biologia dei Sistemi - Life Sciences and System Biology (DBIOS), Turin University, Turin, Italy

CARLO DAGRADI

Dipartimento di Scienze della Vita e Biologia dei Sistemi - Life Sciences and System Biology (DBIOS), Turin University, Turin, Italy

ALESSANDRO GALLO

Dipartimento di Scienze della Vita e Biologia dei Sistemi - Life Sciences and System Biology (DBIOS), Turin University, Turin, Italy

ANNA ZANOLI

Dipartimento di Scienze della Vita e Biologia dei Sistemi - Life Sciences and System Biology (DBIOS), Turin University, Turin, Italy

What do we know about the Ethiopian herpetofauna? Recent distributive atlas dated to 2010 and embraces most of literature and museum specimens starting from mid XIX century to recent times. Recent literature of the last 20-25 yrs covers basically the rediscovery of species considered extinct in the wild (a Ptychadenid frog), description of new taxa (agamid and chameleonid lizards, viperid snake), and the catalogue of Ethiopians snakes. Very little is known in national parks and protected areas and virtually nothing in unprotected areas.

Recent research on Amphibian phylogeny, distribution and conservation underlines that "Taxonomic and biogeographic re-assessment and field surveys in unexplored areas is needed to understand the natural history and population status of Ethiopian amphibians."

Recently we started a long term project on Ethiopian plateau of the Ankober (Ankober, Kundi) and Debre Birhan districts, from 2750 to 3600m asl (260km North Addis). Of 220 reptile and more than 60 amphibian species known, we calculated that potential species could be 10 and 15 respectively (those recorded at altitude >2700m asl). During October 2018 and January-February 2019 we found *Leptopelis gramineus* (Arthroleptidae: 12 sites), *Trioceros affinis* (Chamaeleonidae: 1 site), *Trachylepis megalura* (Scincidae, Mabuyinae: 3 sites), *T. striata* (12 sites), *T. varia* (2 sites), *Philothamnus battersbyi* (Colubridae, Colubrinae: 1 site), *Psammophylax variabilis* (Lamprophiidae, Psammophiinae: 1 site). All of them are new data for Ethiopia and many of them are at higher altitude than that reported in literature.

**POSSIBLE IMPACT OF URBANIZATION: LARGER GREEN TOADS BUT NOT  
ASYMMETRIC IN URBAN AREAS**

MONIKA BALOGOVÁ

Department of Zoology, Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik University, Košice,  
Slovakia

MÁRIA FIGUROVÁ

Small Animal Clinic, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

NATÁLIA PIPOVÁ

Department of Animal Physiology, Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik  
University, Košice, Slovakia

HELENA ULIČNÁ

Department of Zoology, Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik University, Košice,  
Slovakia

MARCEL UHRIN

Department of Zoology, Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik University, Košice,  
Slovakia

The impact of urbanization, nowadays the most common alteration of natural habitats, is still poorly understood in amphibians. In this preliminary study, we observed differences in some morphological patterns and in level of fluctuating asymmetry (FA) in European green toads (*Bufo viridis*) from urban and suburban environment using bone measurements obtained from x-ray scanning pictures. Our results indicated that individuals from urban localities were significantly larger in body length and had longer front/hind limbs than toads from suburban areas. We found significant differences in FA among observed groups in the traits as width of the orbit, femur and calcaneus astragalus bones, while individuals in the urban localities showed surprisingly lower level of the FA in the same parameters than toads from suburban environment. Possible causes of recorded significant differences in morphology and fluctuating asymmetry of individuals from urban and suburban areas are discussed.

**COMPOSITION AND SPECIES RICHNESS OF THE HERPETOFAUNA OF A  
COSTA RICAN UNIVERSITY CAMPUS**

MARCO D. BARQUERO

Sede del Caribe, Universidad de Costa Rica, Limón Costa Rica

ALEXANDER CHAVES

Sede del Caribe, Universidad de Costa Rica, Limón Costa Rica

YORDY SOTO

Sede del Caribe, Universidad de Costa Rica, Limón Costa Rica

JUAN CARLOS SALAS

Sede del Caribe, Universidad de Costa Rica, Limón Costa Rica

The campus of the University of Costa Rica in Limón port city, Costa Rica, known as Sede del Caribe (SC), includes a secondary forest of ca. 40 years old. This forest holds a great potential for environmental education, research, and recreation, although no reliable information on the species present is available. The forest is rarely visited and neither students or staff of SC know the biodiversity of the area. Our aim was to determine the composition and species richness of the herpetofauna present in the campus of SC, in order to provide scientific information and generate educational material that can be distributed to potential visitors. We first collected data on the species of Limón port city area by reviewing the literature. Then, we requested photographs of herpetofauna to students and staff of SC and carried out diurnal and nocturnal samplings to collected data on amphibians and reptiles present in the campus. We have counted 18 amphibians and 42 reptiles inhabiting the campus of SC, representing respectively 25,7% and 37,5% of the species reported for Limón port city. We received a total of 483 photographs from 42 people, 133 photos of amphibians corresponding to 13 species and 350 photos of reptiles corresponding to 25 species. Our samplings produced a total of 15 amphibian and 23 reptile species. These data demonstrate that our project focused the attention of students and staff of the SC towards the herpetofauna of the campus, proving that their collaboration significantly increased our species list. They also indicate that more sampling is required to account for all possible species in the area.



## AN UPDATED ASSESSMENT OF ALIEN WATER FROGS DISTRIBUTION IN ITALY

GIACOMO BRUNI

Viale Togliatti 101, 50019 Sesto Fiorentino, Firenze, Italy

EDOARDO RAZZETTI

Natural History Museum of Pavia, Piazza Botta 10, 27100 Pavia, Italy

IVAN MIRABELLA

Department of Biotechnologies and Sciences, University of Milano Bicocca, Piazza della Scienza 2, 20126 Milano, Italy

SERGIO MEZZADRI

via Palmerio 27/c, 29121 Piacenza, Italy

ANDREA AMBROGIO

via Borghetto 27, 29121 Piacenza, Italy

FRANCESCO PAOLO FARAONE

Viale Regione Siciliana S.E. 532, 90129 Palermo, Italy

CLAUDIA CORTI

Natural History Museum of the University of Firenze, sezione di Zoologia “La Specola”, Via Romana 17, 50125 Firenze, Italy

ANGELA LISA BORGHI

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

MICHELE MAGLIA

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

ADRIANA BELLATI\*

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, 27100 Pavia, Italy

The amphibian genus *Pelophylax* (Anura: Ranidae) is native to Eurasia and Northern Africa and it encompasses approximately 20 cryptic taxa commonly known as water frogs. Among them are included natural hybrid forms named “kleptons” which show a peculiar way of hemiclinal reproduction called hybridogenesis: during gametogenesis one of the parental genomes is discarded prior to meiosis and the remaining is clonally duplicated to produce non-recombined gametes. Kleptons perpetuate themselves through reproduction with the parental species whose genome they discarded during gametogenesis (together constituting a “synklepton”). Native Italian taxa belong to two different synkleptons, respectively: the L-E system of *P. lessonae*/*P. kl. esculentus* widespread in northern Italy, and the B-H system of *P. bergeri*/*P. kl. hispanicus* occurring southwards. Invasive alien populations of the “*ridibundus*” complex have been introduced in Italy since 1940s, but only recently the implementation of molecular diagnostic tools has allowed the detection of multiple cryptic taxa. We update the distribution of alien water frogs across Italy via morphological, bioacoustic and molecular approaches. Genetic characterizations reveal that alien populations mainly belong to the widely translocated *P. ridibundus* (the Marsh frog), the Balkan frog *P. kurtmuelleri*, and the Anatolian frog *P. cf. bedriagae* s.s., with cases of multiple taxa co-occurrence in the same localities. The obtained data add knowledge to the distribution of these widespread invaders in Italy, posing concerns for the conservation of the two native synkleptons.

**IMPACTS OF WINTER TOURISM ON A POPULATION OF *Rana temporaria***

MICHELE CHIACCHIO

UFZ-Helmholtz Centre for Environmental Research; Permoserstr. 15, 04318 Leipzig, Germany

ANNEGRET GRIMM-SEYFARTH

UFZ-Helmholtz Centre for Environmental Research; Permoserstr. 15, 04318 Leipzig, Germany

KLAUS HENLE

UFZ-Helmholtz Centre for Environmental Research; Permoserstr. 15, 04318 Leipzig, Germany

Montane and alpine areas are threatened by the combined effects of land-use and climate change, resulting in the loss of high-altitude natural and historical habitats. Among the sources of landscape modification, ski resorts construction is one of the most intensive and durable, with consequences affecting the environment during both winter and summer seasons. Montane reptiles and amphibians show a high level of specialisation and play a fundamental role in these ecosystems, yet studies on the effects of ski resorts on herpetofauna are rare. Using a dataset gathered in 2018 in Paneveggio-Pale di San Martino Nature Park (Italy), we assessed the consequences of ski-runs management on a population of *Rana temporaria* by measuring individual and population parameters on both ski-runs and meadows. Preliminary results show a difference in the number of individuals between habitats, with a significantly lower number of juveniles and sub-adults being found on ski-runs than on the neighbouring meadows. We also investigated which environmental variables determined species occupancy and found it to be correlated with micro-habitat characteristics such as ground vegetation height and distance from water source. Despite occupancy is not influenced by ski-runs proximity per se, our results suggest that ski-runs management techniques, such as vegetation clear-cutting and mowing, have a negative impact on species occupancy, and can hinder survival of young individuals on ski-runs. Therefore, the implementation of management precautions such as preserving patches of micro-habitats (e.g. tall grasses, shrubs) is recommended for maintaining stable and viable amphibian populations in ski resorts.

**A NEW APPROACH FOR SPATIAL CONSERVATION PLANNING – UNMANNED  
AERIAL VEHICLES IN HIGH RESOLUTION PREDICTIVE POPULATION  
ASSESSMENTS**

VIC CLEMENT

Zoological Research Museum Alexander Koenig, Bonn, Germany

RIEKE SCHLUCKEBIER

Zoological Research Museum Alexander Koenig, Bonn, Germany

JULIA PLATZEN

Zoological Research Museum Alexander Koenig, Bonn, Germany

DENNIS RÖDDER

Zoological Research Museum Alexander Koenig, Bonn, Germany

Fast and efficient data collection has long been a concern in applied ecology and land planning. Our study focuses on developing a method for thorough and efficient spatial explicit population assessment of small vertebrate species in on landscape level. We exemplify our approach using a *Lacerta agilis* population close to Cologne. In a previous study we have performed species distribution models and population connectivity analyses of *Lacerta agilis* populations based on field surveys and remote sensing data. The resulting maps showing potential habitats and gene flow paths are used by an environmental management agency for conservation planning. In our follow up project we expand the data sets and develop much more fine scale predictions for habitat suitability and potential population sizes. One major part of this study is the use of very high resolution remote sensing techniques using a drone, which allow us to quantify not only habitat structure but also microclimatic features. In concert with capture-mark-recapture analyses and radio telemetry, this allows for fine scale assessment of the spatial properties of home ranges and corresponding habitat preference. The study combines conventional field work with novel approaches and has increasing importance nowadays, as detailed small scale ecological assessments are necessary in land use, construction planning and conservation.

**INVASIVE FRESHWATER TERRAPINS IN ABRUZZI AND MOLISE: HISTORY  
OF AN INVASION**

LUCIANO DI TIZIO

S.H.I. Sezione Abruzzo e Molise “Antonio Bellini”, Chieti, Italy

LUCA BRUGNOLA

G.Erp.A.M. – Gruppo Erpetologico Abruzzese e Molisano, Montesilvano (PE), Italy

ANGELO CAMELI

G.Erp.A.M. – Gruppo Erpetologico Abruzzese e Molisano, Montesilvano (PE), Italy

LUCA COPPARI

S.H.I. Sezione Abruzzo e Molise “Antonio Bellini”, Chieti, Italy

Through bibliographic research and an original database, we reported the history of the increasing presence of the invasive species *Trachemys scripta* in two Italian regions, Abruzzi and Molise, starting from the first documented release into the Italian freshwater, reported in 1993 in a locality of these territories.

## **PERSISTENCE AND DYNAMICS OF AMPHIBIAN BREEDING SITES IN IRON GATE NATIONAL PARK, SERBIA**

JELKA CRNOBRNJA-ISAILOVIĆ

Faculty of Sciences and Mathematics University of Niš, 18000 Niš & Institute for Biological Research “S. Stanković” University of Belgrade, 11000 Belgrade, Serbia

DRAGANA MILOJKOVIĆ

Public Company “Djerdap National Park”, 19220 Donji Milanovac, Serbia

Iron Gate refers to transboundary protected areas in Romania and Serbia. On Serbian side, the protected area is considered national park (NP) established in 1974, with altitudes from 45 m to 803 m, mostly forested (70% of the territory). There are also pastures & meadows (10%) and arable land (7%). Only 1% of the land is represented by aquatic ecosystems. Maximal amphibian richness in Iron Gate NP in Serbia was 16 species, with recently confirmed 13 species. A survey conducted in 2012 reported 22 amphibian breeding sites. In 2018, the survey was repeated, resulting in 10 additional breeding sites, two of which (10%) dried out in the late spring. In the second survey, 15 previously recorded sites were checked again for persistence and amphibian presence from beginning of April to the end of May. One site (7%) repeatedly was not active. Amphibian species composition at breeding sites was changing – one site (7%) hosted the same species as seven years ago, at eight sites (56%) the number of amphibian species decreased and at four of them (28%) increased, while at one site (7%) the number of species was the same but qualitative composition changed. Although the survey was limited, the amphibian breeding sites in Iron Gate NP in Serbia could be considered dynamic regarding the number and amphibian species composition hosted in different years.

**AGRICULTURAL LANDSCAPE AND HERPETOFAUNA: A COMPARISON BETWEEN PROTECTED AREAS OF THE ROMAN CAMPAGNA**

PIERANGELO CRUCITTI

Società Romana di Scienze Naturali SRSN, Campus di "Villa Esmeralda", Via Fratelli Maristi 43 00137 Rome, Italy

EDOARDO DI RUSSO

Società Romana di Scienze Naturali SRSN, Campus di "Villa Esmeralda", Via Fratelli Maristi 43 00137 Rome, Italy

CAMILLA FOGGIA

Società Romana di Scienze Naturali SRSN, Campus di "Villa Esmeralda", Via Fratelli Maristi 43 00137 Rome, Italy

AGNESE MARRA

Società Romana di Scienze Naturali SRSN, Campus di "Villa Esmeralda", Via Fratelli Maristi 43 00137 Rome, Italy

NICOLÒ PELLECCIA

Società Romana di Scienze Naturali SRSN, Campus di "Villa Esmeralda", Via Fratelli Maristi 43 00137 Rome, Italy

SAMANTHA ZAGGIA

Società Romana di Scienze Naturali SRSN, Campus di "Villa Esmeralda", Via Fratelli Maristi 43 00137 Rome, Italy

The small natural reserves and parks of the Roman Campagna (established mainly in 1997), north east of Rome's suburban and rural areas, are part of a landscape composed by a mosaic of wood fragments, urbanized and agricultural areas with scattered buildings. Even if these areas are very close to each other (at most a few kilometers as the crow flies), their herpetofauna is quite different, both qualitatively and quantitatively. A project by the Società Romana di Scienze Naturali to record the vertebrate diversity of three protected areas has been in progress for twenty years. Species richness is as follows: Nature Reserve Nomentum with 14 Reptiles and 6 Amphibians; Nature Reserve Macchia di Gattaceca and Macchia del Barco, split in two distinct fragments with respectively 12 Reptiles and 2 Amphibians and 11 Reptiles and 8 Amphibians; the Nature and Archaeological Park of Inviolata with 13 Reptiles and 6 Amphibians. Using the QGIS software, we assigned to each parcels its percentage value of wood, agricultural, still and running water extension. The application of different indexes enabled us to demonstrate the importance of an agricultural landscape in the conservation of this herpetofauna.

## **CURRENT THREATS ON EUROPEAN POND TURTLES (*Emys orbicularis*) AND BALKAN TERRAPIN (*Mauremys rivulata*) FROM VLORA BAY, SOUTHERN ALBANIA**

ENERIT SAÇDANAKU

Research Center of Flora and Fauna, Faculty of Natural Sciences, University of Tirana, Tirana, Albania

This study provides information on current threats for European Pond Turtle (*Emys orbicularis*) and Balkan Terrapin (*Mauremys rivulata*) in the understudied area of Vlora Bay, Albania. Two main types of habitats – freshwater channels (several channels in different areas) and two different ponds were monitored during the period 2013 – 2016. 157 individuals of *Emys orbicularis* and 47 of *Mauremys rivulata* were captured and studied (measured, individually marked, photographed and released again at the point of capture), of which 21 individuals were found dead (14 *E. orbicularis* and seven *M. rivulata*). Mortality rate resulted to be higher in *M. rivulata* population (14.8%; n = 47) in comparison to *E. orbicularis* population (8.9%; n=57). Different threats were identified to cause the death in the population of these two species, based mainly on the signs observed in the dead body of animals. In both populations of *E. orbicularis* and *M. rivulata*, 9.6% of deaths were caused by fragmentation of habitats due to the road infrastructure (death on the roads during female breeding migrations); 19.0% from casual capture at fishing; 33.4% from the different predators attack; and 38% from other causes (human attacks, natural death, etc.). While, other potential threats were identified related to the habitat of the species, as it was the loose and fragmentation of the habitat due to constructions and urbanization; the drying out of ponds and channels during the summer season as well as the interventions and cleaning of the drainage channels.

**IMPACT OF HIGHWAYS ON THE ABUNDANCE OF COMMON CHAMELEON  
(*Chamaeleo chamaeleon*) POPULATIONS.**

MIGUEL-ÁNGEL FARFÁN

Biogeography, Diversity and Conservation research team, Depto. de Biología Animal, Facultad de Ciencias, Universidad de Málaga, E-29071, Málaga, Spain. – BioGea Consultores, Málaga, España

ANTONIO-ROMÁN MUÑOZ

Biogeography, Diversity and Conservation research team, Depto. de Biología Animal, Facultad de Ciencias, Universidad de Málaga, E-29071, Málaga, Spain.

FRANCISCO DÍAZ-RUIZ

Biogeography, Diversity and Conservation research team, Depto. de Biología Animal, Facultad de Ciencias, Universidad de Málaga, E-29071, Málaga, Spain.

ADRIÁN MARTÍN-TABOADA

Biogeography, Diversity and Conservation research team, Depto. de Biología Animal, Facultad de Ciencias, Universidad de Málaga, E-29071, Málaga, Spain.

DARÍO CHAMORRO

Biogeography, Diversity and Conservation research team, Depto. de Biología Animal, Facultad de Ciencias, Universidad de Málaga, E-29071, Málaga, Spain.

JESÚS DUARTE

Ofitecma Marbella SL, Avda. Ramón y Cajal 17, 29601 Marbella, Málaga, España

The need for mobility in modern society has led in recent decades to a considerable increase in infrastructure projects, including motorways and highways. Although roads represent socio-economic development, involve environmental costs. One of the most obvious effects of this type of linear infrastructures on fauna is the fragmentation of the habitat into smaller patches and, consequently, with less capacity to hold viable populations of different species. In this study we analyze the local changes of abundance of common chameleons (*Chamaeleo chamaeleon*) in the province of Malaga due to the presence of the AP-46 highway and the Ronda West of Malaga motorway. To this end, we surveyed 25 squares of 1x1 km, 13 with presence of motorway/hihgway and 12 without presence of these type of road. We calculated in all squares a relative index of abundance per square (IA = number chameleons / hour x beater). The mean IA value in squares with presence of motorway/hihgway ( $0.9 \pm 2.4$ ) was significantly lower than squares without the presence of these linear infrastructures ( $5.0 \pm 8.0$ ) ( $X^2 = 8.000$ ,  $p < 0.01$ ). Although the censuses have not finished yet and, therefore, the results are preliminary, they seem to indicate that the presence of both linear infrastructures have a negative effect on the local abundance of the common chameleon.



**REPRODUCTION IN CAPTIVITY OF THE CAVE NEWT *Proteus anguinus***

OLIVIER GUILLAUME

National Center for Scientific Research, Moulis, France

SUSANNE HOLTZE

Leibniz Institute for Zoo and Wildlife Research, Berlin, Deutschland

ANNE IPSEN

Fledermaus-Zentrum GmbH, Bad Segeberg, Deutschland

*Proteus anguinus* is a salamander that dwells only in subterranean environments in Dinaric karst regions from approximately 20 million years ago. It is certainly the oldest cave dweller of the earth, and now the sole obligate cave Vertebrate of Europe. Little is known about the species in its natural habitat, and most of the knowledge come from captivity. The reproduction outside the Moulis cave is still rare. It seems unpredictable, not at the least seasonal. Sexual maturity, acquired from 15-year-old. We observe only once reproduction per female per 12 years. lifespan is estimated to 68 years and their maximal lifespan to more than 100 years. Actually, understand the reproduction is the key for future conservative programs on this rare species also endangered in nature due to the pollution. For this, the first problem is the sex identification. *Proteus* males and females are indistinguishable by external morphological criteria when they are not sexually active. Also unfortunately, the sex chromosomes of *Proteus* are homomorphic and therefore not useful for sex identification. Here we report some data especially get using ultrasounds technics that improve the knowledge on the reproduction of the species and its management in captivity.

**AMPHIBIAN AND REPTILE ROAD MORTALITY IN THE PROTECTED AREA OF  
OBEDSKA BARA, SERBIA**

MARKO ANĐELKOVIĆ

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

NEDA BOGDANOVIĆ

Faculty of Biology, University of Belgrade, Belgrade, Serbia

GORANA DANON

Faculty of Biology, University of Belgrade, Belgrade, Serbia

MARKO MIRČ

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

TIJANA VUČIĆ

Faculty of Biology, University of Belgrade, Belgrade, Serbia

MAJA AJDUKOVIĆ

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

TANJA VUKOV

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

Millions of animals are roadkilled every year. Amphibians and reptiles are among the most prone to roadkill, especially near ponds and wetlands. We examined the impact of traffic on amphibian and reptile populations on the edge of Special nature reserve “Obedska bara”, Serbia. The aim of our study was to examine the temporal and spatial patterns of amphibian and reptile road mortality. From March to December 2018, we surveyed the road on foot for roadkills on a road section of 4.2 km, with two different habitat types (forest-pond and agricultural area-pond). Within 32 fieldwork days, we recorded 20457 roadkills (nine amphibian and eight reptile species). Amphibians (93%) were more vulnerable to traffic than reptiles (7%). Specimens of *Pelophylax esculentus* complex are the most frequently killed amphibians, followed by *Pelobates fuscus*, while specimens of *Natrix natrix* were the most frequently killed reptile species, followed by *Emys orbicularis*. The numbers of killed amphibians and reptiles varied with the season, indicating that species do not have the same roadkill patterns among the different seasons. Amphibian roadkills were the most frequent during the summer, while reptile roadkills were the most frequent in spring and late summer. Habitat type significantly affected the vulnerability of animals on roads. In addition to the different number of individuals killed in two different habitat types (higher in forest habitat), there was a difference in species composition. This study indicates that detailed monitoring and actions that will mitigate road mortality are urgently needed in the Special nature reserve “Obedska bara”.

## **CONSERVATION OF MOOR FROG (*Rana arvalis*) AND EUROPEAN POND TURTLE (*Emys orbicularis*) HABITATS IN THE LJUBLJANA MOORS, SLOVENIA**

ANJA PEKOLJ

Herpetological Society of Slovenia – Societas herpetologica slovenica, Večna pot 111, 1000 Ljubljana, Slovenia

AJA ZAMOLO

Centre for Cartography of Fauna and Flora, Miklavž na Dravskem polju, Slovenia

Herpetological Society of Slovenia – Societas herpetologica slovenica, Večna pot 111, 1000 Ljubljana, Slovenia

KATJA POBOLJŠAJ

Centre for Cartography of Fauna and Flora, Miklavž na Dravskem polju, Slovenia

Herpetological Society of Slovenia – Societas herpetologica slovenica, Večna pot 111, 1000 Ljubljana, Slovenia

KATARINA DRAŠLER

Herpetological Society of Slovenia – Societas herpetologica slovenica, Večna pot 111, 1000 Ljubljana, Slovenia

GREGOR LIPOVŠEK

Herpetological Society of Slovenia – Societas herpetologica slovenica, Večna pot 111, 1000 Ljubljana, Slovenia

MOJCA VEK

Herpetological Society of Slovenia – Societas herpetologica slovenica, Večna pot 111, 1000 Ljubljana, Slovenia

ANJA BOLČINA

Herpetological Society of Slovenia – Societas herpetologica slovenica, Večna pot 111, 1000 Ljubljana, Slovenia

ANAMARIJA ŽAGAR

Herpetological Society of Slovenia – Societas herpetologica slovenica, Večna pot 111, 1000 Ljubljana, Slovenia

Moor frog (*Rana arvalis*) population in the Ljubljana Moors is isolated for more than 100 km from the next population and thus especially vulnerable. European pond turtle (*Emys orbicularis*), previous surveys have shown that the population structure in the area is consisting mostly of old individuals. For the past six years, the Herpetological Society of Slovenia has run conservation projects in the Ljubljana Moors with regular monitoring for both species. In 2017, we have decided to do a long-term rent of six land parcels (7,2 ha) with the aim to improve the habitat of each species. The land parcels are located in the eastern part of Ljubljana Moors in an area previously covered with an *Alnus glutinosa* forest that has been cut in 2009. Currently it is overgrown with shrubs, small vegetation and alien invasive plants. In October 2018 we have constructed six ponds designed to meet ecological needs of each species. We dug three shallow ponds that are suitable for spawning of Moor frog and three deeper ponds for European pond turtle. The remaining soil from digging was left at the northern side of ponds, creating potential nesting sites for turtles. In the following years we will manage the area and regularly monitor for amphibians and reptiles. We also invited other nature conservation societies to join us in this project of practical conservation and education for our members and wider public. The goal is to monitor currently present flora and fauna which will lead towards the preparation of holistic management plan.

**DISTRIBUTION MODELS OF INVASIVE RED-EARED SLIDER (*Trachemys scripta elegans*) IN THE EASTERN PART OF THE RANGE OF THE SPECIES (UKRAINE AND THE BALTIC COUNTRIES)**

OKSANA NEKRASOVA

Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine

VOLODYMYR TYTAR

Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine

MIHAILS PUPINS

Daugavpils University, Institute of Life Sciences and Technologies, Department of Ecology, Daugavpils, Latvia

The red-eared slider has appeared in many regions of Ukraine due to breeding in captivity. Later the turtles were released into various wetlands. In the last 8 years in the south of Ukraine we have found overwintering turtles. In Latvia since 2007 we have registered more than 40 individuals; the turtles overwinter in Latvia in wild and in an experiment in natural climate conditions. For a closer look into the distribution of the red-eared slider in Ukraine and the Baltic countries, we used GIS-modeling. Species distribution models (SDMs) considering present and future distributions were built using Maxent, DIVA-GIS. As predictors we employed 35 climatic parameters and 5 from the CLIMOND database (Bio36-Bio40). Collectively, they represent a statistical summary of temperature, precipitation, radiation and soil moisture. We used 15,036 Red-eared slider records available through the GBIF, literature and our original findings. Juvenile turtles cannot tolerate mean temperatures below zero. Therefore, there are temperature limitations to the distribution of the species. The most significant factors (according to DIVA-GIS and results of a Factor analysis) are: Annual mean temperature (optimum 6.0–22.7°C), Minimum temperature of coldest month, Mean temperature of coldest quarter preferred above zero. Throughout Ukraine and Latvia abandoned pets are found outdoors, but in the meantime there has been registered no successful reproduction in the countries. The modelling results allowed the identification of suitable habitats for the species in the SW of Ukraine and the Western part of Latvia which will appear in the near future.

**THE LIFE14 NAT/IT/000759 WETFLYAMPHIBIA PROJECT FOR THE  
CONSERVATION OF *Bombina pachypus*, *Salamandrina perspicillata* AND *Triturus  
carnifex* INSIDE FORESTE CASENTINESI NATIONAL PARK**

DAVIDE ALBERTI

Parco Nazionale Foreste Casentinesi, Monte Falterona and Campigna, Pratovecchio, Italy

SANDRO PIAZZINI

Parco Nazionale Foreste Casentinesi, Monte Falterona and Campigna, Pratovecchio, Italy

MATTEO RUOCCO

Dream Italia, Pratovecchio, Italy

The Foreste Casentinesi National Park includes the Apennines ridge between Romagna and Tuscany and can be considered one of the most valuable forest areas in Europe. Its core is represented by the Sasso Fratino Reserve, the first Integral Reserve established in Italy in 1959 recently included in UNESCO World Heritage List, and it also preserves open habitat such as grassland. The National Park is the coordinator beneficiary of the Life project; other beneficiaries are Arma dei Carabinieri, Union of Mountain Municipalities of Casentino, University of Bologna, University of Pavia and Dream Italia. Specific project conservation targets are the improvement of the conservation status of *Bombina pachypus*, *Salamandrina perspicillata* and *Triturus carnifex* and their habitats inside the National Park. *B. pachypus* breeds sometimes in streams flowing in rocky sunny areas, but mostly in small ponds and drinking troughs. It is threatened mainly by the reduction of the reproductive habitats, once common in grazing lands and open areas and has rarefied population in Romagna, but disappeared from the Tuscan side since about 10 years ago. Similarly, *S. perspicillata* is still widespread in Romagna but almost disappeared in the Tuscan side of the National Park. The project proposes some concrete conservation action, such as the restoring actions on wetlands and creation of new ones, the reintroduction of individuals of *B. pachypus* and *S. perspicillata* in conservation areas from the Adriatic to the Tyrrhenian side of the National Park, the production of plants for the botanic restoration of wet habitats.

**EVALUATING NOVEL WETLANDS FOR ASSISTED COLONIZATION OF THE WESTERN SWAMP TURTLE *Pseudemydura umbrina* IN A CHANGING CLIMATE: MACRO-INVERTEBRATE COMMUNITIES, BIOMASS AND TURTLE DIET**

KATJA SCHMÖLZ

University of Vienna, Vienna, Austria and Eurac Research – Institute of Alpine Environment, Bolzano, Italy

ADRIAN M. PINDER

Western Australian Department of Biodiversity, Conservation and Attractions, Wetland Group Kensington, Perth, Australia

GERALD KUCHLING

Western Australian Department of Biodiversity, Conservation and Attractions, Swan Coastal District, Perth, Australia

GÜNTER GOLLMANN

University of Vienna, Vienna, Austria

**Background:** The natural habitat of the Western Swamp Turtle (*Pseudemydura umbrina* Siebenrock, 1901), ephemeral, winter wet swamps north of, Australia, is in danger due to climate change and habitat fragmentation. Rainfall in this area has declined over the last five decades. This trend is predicted to continue, increasingly shortening the annual activity period for the turtles and potentially shifting their optimal climate zone a few hundred kilometers to the south. Assisted colonization to wetlands in the cooler south-west is one management response. As a contribution to such a measure, we undertook a project to evaluate the food availability, biomass and biodiversity at three potential colonization sites.

**Results:** Thirty-five captive-bred juvenile *P. umbrina* were temporarily released at three sites, Moore River Nature Reserve north of Perth, and Meerup and East Augusta in the cooler far south of Western Australia. Both biomass and biodiversity did not differ significantly between the southern sites, and the reference site north of Perth. Prey items were investigated by single flushing of the stomach of each turtle. Stomach contents varied greatly, from 0 to 62 eaten animals per turtle. The main prey items were Coleoptera, Ostracoda, Copepoda and larval Anura.

**Conclusions:** The southern areas may be suitable future habitats for *P. umbrina* in the next decades, as each site provided the invertebrates and vertebrates that the turtles will eat. Assisted colonization into wetlands where *Pseudemydura umbrina* has not been known to occur naturally may become a reasonable conservation tool to ensure the long term survival of this species.

**SQUAMATE REPTILES FROM SEASONAL SEMIDECIDUOUS FOREST  
REMNANTS IN SOUTHWESTERN BAHIA, BRAZIL**

CARLOS AUGUSTO SOUZA-COSTA

Programa de Pós-Graduação em Zoologia, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

MIRCO SOLÉ

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil and Herpetology Section,  
Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

ANTÔNIO JORGE SUZART ARGÔLO

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

We present a list of Squamata from Serra do Mandim and Serra Azul, both in the Atlantic Forest domain of Southern Bahia, Brazil. We recorded 27 species, 21 snakes and six lizards. Most species can be characterized as generalists with a wide distribution, such as *Phyllopezus pollicaris*, *Salvator merianae*, *Corallus hortulanus*, *Philodryas olfersii*, *Oxyrhopus trigeminus* and *Pseudoboa nigra*. However, some of the species are considered as being difficult to sample and restricted to forest fragments such as *Bothrops bilineatus*, *Dipsas sazimai* and *Echivanthera cephalostriata*. The snake fauna of both areas represents 70% of the species previously known for the semideciduous forests of the state of Bahia. Although the study region is under severe anthropogenic pressure, especially due to the expansion of livestock areas, some forest remnants still harbor rich reptile diversity.

**AMPHIBIAN FAUNA IN TWO REMNANTS OF SEMIDECIDUAL SEASONAL FOREST OF SOUTHWESTERN BAHIA, BRAZIL**

CARLOS AUGUSTO SOUZA-COSTA

Programa de Pós-Graduação em Zoologia, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

MIRCO SOLÉ

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil and Herpetology Section,  
Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

ANTÔNIO JORGE SUZART ARGÔLO

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

Amphibian species richness and abundance was studied at two localities of Semideciduous Forest in the municipalities of Potiraguá and Itarantim, both in southwestern Bahia, Brazil. Total species richness was 47 species distributed in 14 families and 26 genera. Approximately half of the species were shared by both areas, while 11 species were only found at the Serra Azul and 10 were exclusive to the Serra do Mandim. A cluster analysis run for 34 localities of the Atlantic Forest, Caatinga and Cerrado using a presence/absence matrix with 190 species revealed that the anuran fauna found at Serra do Mandim and Serra Azul is similar to that found at other localities in northeastern Minas Gerais located in the transitional area between the Atlantic Forest and the Caatinga biomes. Our results show that the remaining forest fragments, even being small and isolated, still harbor a rich anuran diversity, including several Atlantic Forest endemic species as “*Eleutherodactylus*” *bilineatus*, *Phyllodytes luteolus*, *Pristimantis vinhai* and *Oloolygon strigilata* and others considered typical of the Caatinga as *Leptodactylus troglodytes* and *Physalaemus cicada*.



**SYNERGISTIC EFFECTS IN NATURAL HISTORY AS PREDICTORS OF  
VULNERABILITY TO GLOBAL WARMING IN AMPHIBIANS**

LEILDO M. CARILLO FILHO

Universidade Estadual de Santa Cruz, Ilhéus, Brazil

BRUNO T. DE ORRICO

Universidade Estadual de Santa Cruz, Ilhéus, Brazil

BRUNA K. A. AZEVEDO

Universidade Estadual de Santa Cruz, Ilhéus, Brazil

LUIS M. GUTIÉRREZ-PESQUERA

Department of Evolutionary Ecology, Estación Biológica de Doñana, CSIC, Sevilla, Spain

CAIO V. MIRA-MENDES

Universidade Estadual de Santa Cruz, Ilhéus, Brazil

MIRCO SOLÉ

Universidade Estadual de Santa Cruz, Ilhéus, Brazil

VICTOR G. D. ORRICO

Universidade Estadual de Santa Cruz, Ilhéus, Brazil

While an estimated 200 amphibian species worldwide may already have gone extinct, the fate of many others is still uncertain. In the Brazilian Atlantic Forest (AF) amphibians face degradation of their habitats and exposure to stressful thermal conditions. We evaluated the thermal tolerance of 47 amphibian species from the AF by estimating the maximum critical temperature (CT<sub>Max</sub>) and warming tolerance (WT). We incorporated ecophysiological indexes (CT<sub>max</sub> and WT), ecological data (e.g. environments used by the adults, tadpole development sites) and meristic morphological characters in a selection of Phylogenetic Generalized Least Squares model (PGLS) using a Brownian motion model. Although CT<sub>Max</sub> was not predicted by the evolutionary model in our analyses, our results point to a strong association between CT<sub>Max</sub> and the environment (adult habitat and/or tadpole development environment). Species from open environment and those considered generalists were more heat tolerant than those typically associated with forests. Species with lentic larval development had higher tolerances to elevated temperatures than those from lotic environments. Thus, forest species, dependent on microclimates, would be more threatened by habitat loss and global warming. We highlight a probable thermal split between the adult habitat and that used by tadpoles, especially for forest lineages. Although many species do not experience environmental temperatures outside their tolerable limits, the exposure of microclimatic refuges through deforestation can result in thermal situation totally unfavourable to the conservation of the species.

**CASE OF BRUMATION AND PREDATION OF A TRAPPED, RELEASED AND  
FISHED INVASIVE ALIEN TURTLE DOCUMENTED BY TELEMETRY AND  
FORENSIC NOTES IN EASTERN POLAND**

DANIELE MARINI

Former Erasmus+ Trainee of Epicrates Foundation & National Veterinary Research Institute, Lublin & Puławy, Poland

ANNA ZIĘTEK-BARSZCZ

Department of Epidemiology and Risk Assessment, National Veterinary Research Institute, Puławy, Poland

BARTŁOMIEJ GORZKOWSKI

Epicrates Foundation, Lublin, Poland

MAGDALENA ZAJĄC

Department of Microbiology, National Veterinary Research Institute, Puławy, Poland

DARIUSZ WASYL

Department of Microbiology and Department of Omics Analysis, National Veterinary Research Institute, Puławy, Poland

KRZYSZTOF ŚMIETANKA

Department of Poultry Disease, National Veterinary Research Institute, Puławy, Poland

On 10<sup>th</sup> July 2016, a *Trachemys scripta elegans* was captured with Epicrates Turtle Trap settled out in Zalew Zemborzycki, Lublin. Slider, showing good condition, was offered to telemetry task. GPS/GSM transmitter was fastened on carapace and, on 18<sup>th</sup> August 2016, turtle was released at original trapping site. To geolocalize position and track migration paths, signals were sent to Ecotone Software and were transferred to maps using Google Earth Pro. Till 11<sup>th</sup> October 2016 signals from turtle showed great mobility across lake. Next year, from 18<sup>th</sup> March to 20<sup>th</sup> March 2017 transmitter started to send signals showing great movements, but it sent signals till the next two following days from mainland and not from the shore. Signals were retransmitted from this last location from 12<sup>th</sup> April 2017 till visit of authors. On 20<sup>th</sup> April the carcass of the slider was found in the area where signals were sent from 21<sup>th</sup> March: the cadaver lay on undergrowth showing gnawing traumas and absence of all limb extremities, the skull distant few meters with a hook deeply embedded in oropharynx with many meters of wire. The inspection showed necrophilous Diptera larvae and Silphidae mating on the carcass. Comparison of temperatures from transmitters and environment were congruent and the activity registered from 21<sup>th</sup> March 2017 was close to 0. Telemetry of slider confirms brumation of invasive alien turtles in Lublin Region and let hypothesized that, awakening from brumation, the slider was fished on baited set line and, then, an easy prey for some predator.

**POPULATION RESPONSE OF BRAZILIAN LIZARDS TO FOREST COVER  
REDUCTION AT THE LANDSCAPE SCALE, AND PRESENCE OF EXTINCTION  
THRESHOLDS**

MARLLA ALVES MATOS

Centro de Conservação e Manejo de Fauna da Caatinga, Universidade Federal do Vale do São Francisco, Petrolina, Brazil

EUVALDO MARCIANO-JR

Centro de Conservação e Manejo de Fauna da Caatinga, Universidade Federal do Vale do São Francisco, Petrolina, Brazil

GEANE LIMEIRA DA SILVA

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

MIRCO SOLÉ

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil and Herpetology Section,  
Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

Habitat loss caused by human activities is pointed as main driver of biodiversity reduction worldwide. Recent studies propose the existence of extinction thresholds to explain the response of species to forest cover reduction in the landscape. These studies also suggest critical values of habitat loss from which on abrupt reduction in the number of individuals of a population would occur. Here, we assessed the response of three forest lizard species (*Coleodactylus meridionalis*, *Enyalius catenatus* and *Gymnodactylus darwini*) from the Brazilian Atlantic Forest to habitat loss by using model selection. Sampling was carried out in nine landscapes from the bahian Atlantic Forest in 2011. The forest cover in the landscapes ranged from 5% to 55%. We found different responses for each species. The null model fitted to *E. catenatus*, while linear and non-linear models fitted to *C. meridionalis* and *G. darwini* data, respectively. Thus, we suggest that forest cover reduction in the landscape scale is a threat to the populations of the two latter species. Despite of the three species being forest dependant species, the different response to cover reduction may reflect their distinct ecological requirements, as specificity of micro-habitat. More studies are needed to include other variables of interest.

**MOORLANDS - SPECIAL HABITATS FOR AMPHIBIANS AND REPTILES:  
DIVERSITY, DISTRIBUTION, HABITAT USE AND CONSERVATION IN THE  
“WALDVIERTLER MOORLANDSCHAFT” NATURA 2000 SITE**

SUSANNE STÜCKLER

Department of Integrative Zoology, University of Vienna, Althanstraße 14, 1090 Vienna, Austria.  
First Zoological Department, Herpetological Collection, Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria

WALTER HÖDL

Department of Integrative Zoology, University of Vienna, Althanstraße 14, 1090 Vienna, Austria.

SILKE SCHWEIGER

First Zoological Department, Herpetological Collection, Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria

Moors are important ecosystems with essential tasks such as storing water, carbon and nutrients in high amounts. These sensitive ecosystems provide habitats for numerous species, whereby many of them show high levels of specialization. Industrial peat extraction, forestry and the accompanying drainage endanger these ecosystems. In the Lower Austria's Waldviertel region, many moors are part of the NATURA 2000 Network and the Ramsar Convention, and therefore gradually undergo restoration measures to recover from destruction. These moors shelter amphibians and reptiles, whereby many of them are on the IUCN Red List of threatened species and in Appendix II of the Habitats Directive.

Our study focuses on the herpetofauna of four different moors located in the Waldviertel region in Lower Austria. We aimed to investigate which amphibian and reptile species inhabit the moorlands and to characterize available microhabitats. Further, we discuss if the protection and restoration of the moorlands also protects the amphibian and reptile populations. Additionally, as there is only little data available, this study will serve as a baseline inventory of the herpetofauna. We combined different methods to assess the herpetofauna. To map the microhabitats and to investigate which factors may influence the species diversity, we conducted a mapping of the landscape structure and the habitat use.

In total, we found nine amphibian and four reptile species in the four moorlands. The results of the study show that the Natura 2000 site provide habitats for amphibians and reptiles, species that are threatened all over in the cultural landscape.

**THE AUSTRIAN ARMED FORCES – POSSIBLE IMPAIRMENTS BY THE  
HERPETOFAUNA IN OPERATIONAL AREAS OF AFRICA**

SILKE SCHWEIGER

First Zoological Department, Herpetological Collection, Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria

FLORIAN BACHER

First Zoological Department, Herpetological Collection, Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria

KARIN ERNST

First Zoological Department, Herpetological Collection, Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria

SUSANNE STÜCKLER

First Zoological Department, Herpetological Collection, Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria

CHRISTOPH PLUTZAR

Institute for Social Ecology, University of Natural Resources and Life Science, Schottenfeldgasse 29, 1070 Vienna, Austria  
Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, 1030 Vienna, Austria

During military missions abroad in conflict areas of West and East Africa, potentially dangerous species can pose unexpected threats to soldiers. Animal-based risks are mosquito-borne diseases, e.g. Malaria or yellow fever, as well as snakebites. The aim of the project was to establish a database with detailed biological and ecological information of reptile species in order to derive operation-relevant aspects for study areas in West and East Africa (Gambia, Mali, Mauritania, Senegal, Ethiopia, Eritrea, Somalia, Sudan). Information on potentially dangerous reptile species like Vipers, Elapids and opisthoglyphous snakes, which contain strong potential hazards, as well as crocodiles and monitor lizards was researched. Online databases, scientific publications and the collections of the Natural History Museum of Vienna were used as data sources. It turned out that some study areas are barely explored, or literature is hardly available. All data was transferred into a geographic information system (GIS) to create distribution maps of potentially dangerous snakes, hotspot maps and maps showing the locations of medical facilities. For the study area in West and East Africa, a total of 163 records were created. In order to enable a possible determination of potentially dangerous snake species in the operational areas in West and East Africa, two identifications booklets were created which include additional information about first aid in case of, and precautionary measures designed to prevent snakebites.

**TOWARDS IMPORTANT HERPETOFAUNA AREAS (IHA'S) IN EUROPE**

RONALD ZOLLINGER

RAVON, Nijmegen, Netherlands

TONY GENT

Amphibian and Reptile Conservation Trust, Bournemouth, UK

JIM FOSTER

Amphibian and Reptile Conservation Trust, Bournemouth, UK

The identification of important areas for biodiversity, and specifically for different taxa (e.g. birds, plants), is an approach that has been used effectively for directing conservation action and for ensuring sufficient site safeguards. It is also a valuable mechanism for ascertaining whether sufficient conservation attention is being given to the most significant biodiversity hot spots. An attempt to develop a European inventory of Important Herpetofauna Areas was initiated in the early 2000s with Alterra; this developed a methodology, an on-line reporting system, and a network of compilers. However very few areas were identified and the project suffered from little take up. Nonetheless, the ambition to develop such a project remains both in a pan-European context and at national levels. This will help allow an assessment of how well the protected area series, notably Natura 2000 and Emerald network sites, cover the key herpetofaunal sites and also the degree to which the conservation objectives of these sites address herpetofauna conservation needs. Through Reptile and Amphibian Conservation Europe (RACE) we have started a new project by which Important Herpetofaunal Areas are identified and mapped. This will review previous work on the topic and look at approaches used for different taxa, identify objectives, criteria and determine how these areas can be used to further conservation; it will develop the mechanism for identifying the sites, the contributors and means for analysis and dissemination. This poster is intended to encourage cooperation in the project.

**FIRST RECORD OF *Mesocestoides litteratus* IN A WALL LIZARD (*Podarcis siculus*)  
FROM CENTRAL ITALY**

EMANUELE BERRILLI

Università degli studi dell'Aquila, L'Aquila, Italy

ALESSANDRA RICCIERI

Dipartimento di Scienze, Università Roma Tre, Rome, Italy

GIULIA SIMBULA

Dipartimento di Scienze, Università Roma Tre, Rome, Italy

The Italian wall lizard, *Podarcis siculus*, is one of the most common lizards in Italy, but is poorly investigated from a parasitological perspective. In this study we report the first evidence of parasitic infection by second larval stage (tetrathyridium) of the genus *Mesocestoides* (Cestoda, Mesocestoididae) in a wall lizard collected in central Italy. The complete life cycle of this tapeworm is not clear yet, but some reptiles, amphibians, birds and micro-mammals have been indicated as second intermediate hosts. Morphological identification at species level of this parasite is extremely difficult, and the taxonomy of *Mesocestoides* is still largely debated. Several species have been described, but only four are considered valid. In this study we aimed to identify the tetrathyridia and to assess their phylogenetic position using the barcode region COI. Molecular identification using the Basic Local Alignment Search Tool (BLAST) assigned the isolates from the lizard to *Mesocestoides litteratus* and Bayesian and Maximum Likelihood results confirmed their phylogenetic position nested inside the clade of this species. Until now, *M. litteratus* has been observed mainly in wild and domestic carnivores as dogs, cats and foxes, which represent the definitive hosts. In conclusion, according to our finding, *P. siculus* seems to have an important role as second intermediate host in the transmission route of this tapeworm species infection.

## MICROBIAL COMMUNITY OF GREEN FROG'S SKIN

KATARINA BREKA

University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia, [katarina.breka@bio.bg.ac.rs](mailto:katarina.breka@bio.bg.ac.rs)

MILOŠ STUPAR

University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden „Jevremovac“, Belgrade, Serbia

DANIJELA VIDAKOVIĆ

University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia

MILICA LJALJEVIĆ GRBIĆ

University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden „Jevremovac“, Belgrade, Serbia

JELENA KRIZMANIĆ

University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden „Jevremovac“, Belgrade, Serbia

IMRE KRIZMANIĆ

University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia

SRĐAN STAMENKOVIĆ

University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia

Amphibian populations have been declining throughout the world in the last several decades. Although numerous factors, including habitat destruction, pollution, introduced species, and global environmental changes contribute the most to amphibian population decline, yet many amphibians are dying of infectious diseases.

Recent studies of amphibian skin infections were mainly focused on the presence of chytridiomycosis neglecting other members of the frogs' skin communities. Samples for mycological and algological analyses were collected from 100 individuals belonging to *Pelophylax esculenta* complex on three localities in South Banat, Serbia using non-aggressive, rapid and inexpensive “adhesive tape” method for microbial biofilm observation.

In analysed samples variety of fungal reproductive structures, and in few samples spore germination, and mycelia formation were detected. Sporulation of some typical soil-borne fungi and the causative agent of amphibian chromomycosis as well as human phaeohyphomycosis, *Fonsecaea* sp. was detected along with water mold, *Aphanomyces* sp. the pathogen responsible for amphibian egg mortality and saprolegniasis of mostly salamanders and tadpoles. For both pathogens, these were the first reports for green frogs in Serbia. Microscopy analysis showed the presence of aeroaquatic hyphomycetes (eg. *Canalisporium* sp.). Besides mycobiota, the frog's skin has proved to be a good medium for the survival of diatoms (detected in high diversity and abundance), Cyanobacteria and Chlorophyta.

Adhesive tape method proved itself useful as a consequential diagnostic tool for preliminary observation of the microbial community on the skin of amphibians including potential pathogens and symbionts. It provides minimal stress to the studied animal and can be easily applicable in various conditions of field research.



**PILOT SURVEY ON THE AMPHIBIAN CHYTRID FUNGI IN SLOVENIA**

MOJCA VEK

Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

MARTINA TURK

Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

NINO KIRBIŠ

*Societas herpetologica slovenica*, Ljubljana, Slovenia

KATJA POBOLJŠAJ

Centre for Cartography of Fauna and Flora, Miklavž na Dravskem polju, Slovenia

*Societas herpetologica slovenica*, Ljubljana, Slovenia

GREGOR ALJANČIČ

Tular Cave Laboratory, Society for Cave Biology, Kranj, Slovenia

NINA GUNDE CIMERMAN

Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

ROK KOSTANJŠEK

Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Amphibian chytridiomycosis is a worldwide-spread infectious disease caused by pathogenic fungi *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*). Due to its quick spread, together with high infectivity and mortality, chytridiomycosis is responsible for a major amphibian biodiversity loss on a global scale over recent decades. Whereas *Bsal* has a relatively localized distribution in Europe, the presence of *Bd* has been confirmed in 12 European countries. Here we present the first results of a pilot survey on chytrid fungi in Slovenia, conducted in 2017 within the project “Invasive species never rest”. Our survey included swab samples of ventral body side and extremities from 52 individuals belonging to 12 amphibian species from selected parts of Slovenia. The DNA isolation and standardized qPCR-based diagnostics of samples was conducted at the Department of Biology, Biotechnical Faculty, University of Ljubljana, where detailed studies on the amphibian diseases are carried out. No infection with *Bsal* or *Bd* has been detected during the study. Our findings do not allow the inference on the absolute absence of the *Bd* and *Bsal* in Slovenia, as a regular national screening on the disease is needed.

**AN ALIEN IN MY POND! INNATE BEHAVIOURAL RESPONSES TOWARDS AN  
INVASIVE PREDATOR IN LARVAE OF NORTHERN ITALY AMPHIBIAN  
COMMUNITY**

ELISA ALARI\*

Department of Environmental Sciences and Policy, University of Milan. Via Celoria 26, 20133 Milano.

SAMUELE ROMAGNOLI

Department of Environmental Sciences and Policy, University of Milan. Via Celoria 26, 20133 Milano.

GENTILE FRANCESCO FICETOLA

Department of Environmental Sciences and Policy, University of Milan. Via Celoria 26, 20133 Milano.

RAOUL MANENTI

Department of Environmental Sciences and Policy, University of Milan. Via Celoria 26, 20133 Milano.

ANDREA MELOTTO

Department of Environmental Sciences and Policy, University of Milan. Via Celoria 26, 20133 Milano.

\* Corresponding author:

Email address: [elisa.alari@studenti.unimi.it](mailto:elisa.alari@studenti.unimi.it)

Failure to express effective responses towards invasive alien predators constitutes an important driver of decline in native species and is generally attributed to their lack of common evolutionary history with invasive species. However, mechanisms allowing innate recognition of invasive predators exist. Innate responses to invasive predators are heterogeneous among species and their occurrence can strictly depend on ecology and prey evolutionary history. The present study provides an overview on the behavioural responses in larvae of the amphibian community of Northern Italy (i.e. 8 anuran and 5 urodele species) towards the invasive crayfish *Procambarus clarkii*, which is a voracious and widespread invasive predator, often associated to amphibian declines. We collected amphibian larvae from uninvaded sites, and assessed the occurrence of anti-predator behaviour by exposing them to both visual and chemical cues of the invasive predator, evaluating their activity and space use.

The larvae of most species modulated their behaviour in response to both chemical and visual cues of the alien predator, while modality and intensity of anti-predator behaviour strongly differed among different species. The response to visual cues was generally consistent among species, whereas responses to chemical showed more complex patterns. Our results suggest that amphibian larvae can recognise the invasive crayfish as a threat and express general anti-predator behaviour. Even if the effectiveness of these responses in wild conditions has still to be cleared, these data together with field surveys can contribute to understand native amphibian population trends in invaded areas.

**SEXUAL SIZE DIMORPHISM AND ECOMORPHOLOGY OF THE  
BERTHOLD'S BUSH ANOLE (*Polychrus guttuerosus*) IN COSTA RICA:  
PRELIMINARY DATA**

VIVIANA ARGUEDAS

Recinto de Paraíso, Sede del Atlántico, Universidad de Costa Rica, Cartago, Costa Rica

MARCO D. BARQUERO

Sede del Caribe, Universidad de Costa Rica, Limón, Costa Rica

RANDALL ARGUEDAS

Zoológico Nacional Simón Bolívar, San José, Costa Rica

LISBETH OVARES

Zoológico Nacional Simón Bolívar, San José, Costa Rica

RODOLFO VARGAS

Refugio Herpetológico de Costa Rica, San José, Costa Rica

Sexual size dimorphism (SSD) is the result of sexual or natural selection operating differently on the body sizes of females and males. Males of most lizard species are larger than females, suggesting intrasexual selection on male's body size to be more aggressive or territorial. However, some species exhibit female-biased SSD, suggesting that selection operates on female fecundity. A third explanation for SSD concerns with differences among sexes to reduce intersexual competition through a differential resource use. The Berthold's bush anole is a rare arboreal and diurnal lizard, for which there is little information about its ecology and behavior. Our aim is to determine whether morphological and ecological measures differ across sexes and relate potential differences with hypotheses explaining SSD. We studied one population of the Berthold's bush anole in Costa Rica. We collected data on eight morphological measurements, skin temperature and sleeping perch height. We captured and pit tagged 35 wild individuals, 18 males and 17 females. We found that females are on average 23,1 mm larger than males, but relative tail length, head length and head height are male-biased. We found no differences in the sleeping perch height among sexes and did find a negative correlation between sleeping perch height and nocturnal skin temperature for females but not for males. These preliminary results demonstrate that female-biased SSD is present in the Berthold's bush anole, but only in SVL. They also seem to indicate that either a fecundity or ecological hypothesis might explain such SSD, although further research is underway to explore these options.

**PREDATION ON *Mauremys leprosa* ATREGHAIA LAKE IN NORTHERN ALGERIA**

BENEBRI IMENE

Laboratory of Dynamics and Biodiversity, Houari Boumediene University, Algiers, Algeria.

CHERGUI KHADIJDA

Laboratory of Dynamics and Biodiversity, Houari Boumediene University, Algiers, Algeria.

BAKHOUCHE BADIS

Laboratory of Dynamics and Biodiversity, Houari Boumediene University, Algiers, Algeria

GHOULAM TIAR

Department of Biology, Faculty of Natural Sciences and Life, University Chadli Bendjedid, El Tarf, BP 36100 El Tarf, Algeria.

Research Laboratory "Ecology of Terrestrial and Aquatic Systems", University Badji Mokhtar, Annaba, Algeria

DINO SCARAVELLI

Department of Veterinary Medical Science, University of Bologna, via Tolara di Sopra 50, 40064 - Ozzano dell'Emilia (BO), BOLOGNA, ITALY

The Mediterranean Pond Turtle *Mauremys leprosa* (Schoepff in Schweigger 1812) is a freshwater turtle with distinctive yellow -orange stripes on neck and limbs typical of the humid zone of the western Mediterranean region. As other Geoemydids, it can be predated, especially at a young age, by a certain number of terrestrial and winged predators, which can in some cases threaten survival when small isolated populations are considered.

Aims of the study was to investigate the possible pressure by predation on the population of *M. leprosa* in the Reghaia lake in northern Algeria, in order assess possible threats and promote turtle conservation.

Forty-six turtle shells were collected on shore of the lake and 24 show clear signs of predation as signs of bites and deep erosions in the back of the carapace. Thirteen Jackal dungs collected close to dead turtles and at nest sites contained bones and remains of carapace of *Mauremys*.

Among the 24 carapaces found, 13 belonged to females and 11 to males. In parallel we captured 47 live turtles, 30 males, 10 females and 7 juveniles. The 14.9% of specimens presented mutilated hind limbs (80% male and 20% female). Females seems to be a little more susceptible to predation, representing the 54.16% of the cases.

The role of predation in the conservation of this isolated population is also discussed.

**NOTES ON THE DIET OF *Mauremys leprosa* IN REGHAIA LAKE (RAMSAR SITE, NORTHERN ALGERIA)**

CHERGUI KHADIJDA

Laboratory of Dynamics and Biodiversity, Houari Boumediene University, Algiers, Algeria.

BENEBRI IMENE

Laboratory of Dynamics and Biodiversity, Houari Boumediene University, Algiers, Algeria.

BAKHOUCHE BADIS

Laboratory of Dynamics and Biodiversity, Houari Boumediene University, Algiers, Algeria

CHAFFAI AMIN

Laboratory of Dynamics and Biodiversity, Houari Boumediene University, Algiers, Algeria

DINO SCARAVELLI

Department of Veterinary Medical Science, University of Bologna, via Tolara di Sopra 50, 40064 - Ozzano dell'Emilia (BO), BOLOGNA, ITALY

Freshwater turtles are still poorly known in Algeria, threatened by non-conservative use of ponds, lakes and wadis. The study was focused on the determination of the diet of Mediterranean turtle *Mauremys leprosa* in Lake Réghaia (northern Algeria). Indeed, this turtle is a typical species of the wetlands of the Mediterranean zone, where it mainly inhabits the different wadis with semi-permanent waters.

The study was conducted from April to November 2018 and was based on stomach washing of individuals caught by hand on the shores of the lake. Thirty-two individuals, 23 males, 3 females and 6 youngs, were examined. Nineteen stomachs were empty and food items were found in 13, corresponding to an annual emptiness coefficient of 59.37%. As attended, in summer the emptiness was the lower (28.6%), increasing in spring (66.7%) and autumn (69.2%).

The collection of the stomach contents shows a large spectrum mostly focused on insects, both larvae and adults, but also comprising fish carcasses and plants.

On 139 items found, just six (4.3%) were not possible to identify as small animal's parts. The larger category was represented by insects with the 87% of items. Adults of different orders were 16 (11.5%) and larvae 105 (75.5). Mosquitos are the main group of prey with a cumulative percentage of the 38.8%. Also Crustaceans were the 1.4% and plant parts constituted the 6.5%. Plant materials are actually found only in the small sample of females.

Their opportunistic predation can be also considered an important factor of mosquito control.

## FEEDING HABITS OF THE WATER GREEN FROGS (*Pelophylax esculentus* COMPLEX) IN R-E-L POPULATION SYSTEMS

KATARINA BREKA

University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia, [katarina.breka@bio.bg.ac.rs](mailto:katarina.breka@bio.bg.ac.rs)

MILAN PLEČAŠ

University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia

BORIS DUDIĆ

University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia

KATARINA STOJANOVIĆ

University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia

NIKOLA VESOVIĆ

University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia

IMRE KRIZMANIĆ

University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia

SRĐAN STAMENKOVIĆ

University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia

Population systems where all three taxa of *Pelophylax esculentus* hybridogenetic complex coexist are present in few areas in Serbia, including the South Banat which is the southern geographic range limit of *P. lessonae*, one of the parental species. Although listed as Least Concern at the global scale, as peripheral populations are often less abundant and more vulnerable, *P. lessonae* could be potentially considered endangered on a regional level since it is listed as Data Deficient for Serbia.

During three years (2016-2018), we examined the diet of *Pelophylax esculentus* complex in three localities with different levels of anthropogenic pressure. Frogs were identified to species level based on selected qualitative and quantitative traits (Krizmanic, 2008). We obtained diet samples from 317 adult frogs (*P. kl. esc* – 226; *P. rid* – 62; *P. les* - 29) using the stomach flushing method and 1492 diet items were identified to order level. The diet consisted mostly of adult terrestrial arthropod prey (87.87%). The dominant prey groups in all three taxa were Hymenoptera (27.68%), Coleoptera (17.96%), Lepidoptera (16.76%) and Diptera (10.66%). Other groups had representation below 10% which indicated opportunistic prey selection behavior in line with optimal foraging theory and previous studies.

The average frog consumed 6.75 prey items, of 10.35 mm average length and 837mm<sup>3</sup> volume. The average trophic niche width was 0.31. In accordance with their sizes, the largest average prey was recorded for *P. ridibundus* and smallest for *P. lessonae*. At each locality, *P. lessonae* had the narrowest niche (0.04-0.14) while *P. ridibundus* had the widest (0.25-0.32). Among localities, *P. lessonae* had the widest (0.41) and *P. ridibundus* the narrowest niche (0.14). Simpson's diversity index showed *P. lessonae* had the highest prey diversity of taxa (0.74). The narrow niche of *P. lessonae* on each locality and the widest across localities may indicate trophic niche specialization since it used different categories of prey on each site in relation to *P. ridibundus*. Based on prey composition, the hybrid taxon was more similar to *P. lessonae*. Although it was expected that the highest prey diversity would be in the more natural areas, the results indicate that it is in areas with higher anthropogenic activity.

## ECOLOGICAL NOTES ON THE SPUR-THIGHED TORTOISE IN ITALY

CLAUDIA CORTI

Museo di Storia Naturale dell'Università degli Studi di Firenze, Sede "La Specola", Via Romana 17, Firenze, Italia

MARTA BIAGGINI

Museo di Storia Naturale dell'Università degli Studi di Firenze, Sede "La Specola", Via Romana 17, Firenze, Italia

*Testudo graeca* Linnaeus, 1758 is present in Italy with viable populations in central-western Sardinia. Classified as Near Threatened in the IUCN Italian Red List assessment, this species is mainly threatened by human-induced land-use changes (in particular in agricultural habitats) and by illegal collection and translocations and related consequences (e.g., possible spread of infectious diseases). Here we report field observations on the activity pattern and thermal ecology of an Italian Spur-thighed tortoise population inhabiting a coastal region characterized by open uncultivated areas and Mediterranean maquis. We focused on the two seasons of higher activity: spring and autumn. Indeed, a relatively intense activity, including courtship behavior, was observed in November. Analyzing the pattern of occurrence of tortoise behaviors in relation to environmental temperatures, we observed low variability. We did not record significant differences among temperatures at which behaviors were displayed and males and females showed comparable patterns.

## **TERRITORIAL RESPONSE TO VISUAL STIMULI IN *Podarcis* LIZARDS**

ARIS DEIMEZIS-TSIKOUTAS

Section of Zoology and Marine Biology, Department of Biology, National and Kapodistrian University of Athens, Athens, Greece

AIKATERINI-DIMITRA PITSIKA

Section of Zoology and Marine Biology, Department of Biology, National and Kapodistrian University of Athens, Athens, Greece

NATASA BELLOU

Section of Zoology and Marine Biology, Department of Biology, National and Kapodistrian University of Athens, Athens, Greece

PANAYIOTIS PAFILIS

Section of Zoology and Marine Biology, Department of Biology, National and Kapodistrian University of Athens, Athens, Greece

Intraspecific aggression is very common in territorial lizard species, as individuals have to compete for resources and access to the best locations in terms of food availability, shelter and thermal quality. Environmental perception is based primarily on chemical cues, but visual and auditory stimuli are also employed. Visual communication is important in territorial challenges and its role remains to be fully understood and evaluated. Here, we aimed to test the territorial responses elicited by a visual stimulus in seven *Podarcis* species. We hypothesised that species living in dense populations would have adopted different behaviours and respond differently to visual stimuli, compared to their congenics that live in low population densities. In the laboratory, we used an arena fitted with a mirror on one side and recorded the lizards' reactions to their mirror idol. Control trials were also executed by covering the mirror with a cardboard. The observed behaviours were scored depending on the level of asserted aggression. Thus, highly aggressive behaviours, such as attempted bites or lunges towards the idol received higher score points than behaviours showing mere interest in the idol or aggressive displays that did not lead to attacks. In this way, total scores were computed for each individual. Our results rendered partial support to our initial hypothesis. Lizards deriving from denser populations tend to adopt milder behaviours and respond in a lower percentage to visual stimuli. On the contrary, lizards from more scarce populations were more aggressive towards conspecifics. However, this was not a universal pattern and exceptions were recorded.



**HABITAT PREFERENCES OF THE COMMON CHAMELEON (*Chamaeleo chamaeleon*) IN SAMOS ISLAND, GREECE**

FRANCISCO JAVIER FALQUINA

Archipelagos Institute of Marine Conservation, Samos, Greece

ANA LIS CORES

Archipelagos Institute of Marine Conservation, Samos, Greece

GUIDO PIETROLUONGO

Archipelagos Institute of Marine Conservation, Samos, Greece

JASON NAGRO

Archipelagos Institute of Marine Conservation, Samos, Greece

ANASTASIA MILIOU

Archipelagos Institute of Marine Conservation, Samos, Greece

Samos Island, in the Eastern Aegean Sea, hosts the largest population of the Common chameleon (*Chamaeleo chamaeleon*) species in Greece. The EU Habitats Directive list its conservation status as “unfavorable-inadequate” and the 65.5% of the island is considered as unsuitable habitat for it. The objectives of the research are to showcase the chameleon habitat preference and their adaptation strategies to anthropogenic environmental changes. Data were collected in 2016 and 2018. The study was located in Southeastern Samos. Five different habitats (mixed forest, coastal, riverbed, olive grove and marsh) were determined based upon the macrohabitat level and their proximity to anthropogenic factors. Additionally, individual microhabitat and morphometric variables were recorded. A total of 157 *C. chamaeleon* were observed: 79 adults, 16 juveniles, 44 newborns and 18 unknowns. Most observations occurred from July to September. No significant differences in sex ratio nor snout-ventral length were found. Chameleons showed a preference for dense vegetation and relatively tall plants (3.34 m mean height), perch height between 1-2 m and for the external part of branches. Newborns and juveniles were observed in the herbaceous and bushy states whilst adults were in bushes and trees. Riverbed with *Vitex agnus-castus*, *Pistacia lentiscus* and *Platanus orientalis* as dominant plant species was the habitat with the highest chameleon presence. This study reveals the preferred habitats of the *C. chamaeleon* population in Samos Island and highlights the importance of preserving these specific environments to minimize the threats on this peculiar native population.

***Rana temporaria* MATING CALLS VARIATION TO DESIGN CONSERVATION  
UNIT IN NORTHERN APENNINE**

BENEDETTA GAMBOLI

Scuola di Agraria e Veterinaria, University of Bologna, Italy

PAMELA PRIORI

Museo Ornitologico F. Foschi, Forlì, Italy

DINO SCARAVELLI

Department of Veterinary Medical Science, University of Bologna, Italy dino.scaravelli@unibo.it

Bioacoustics in anurans can serve as an important tool for both eco-ethological investigations and species identification. The advertisement call has been described as the most important sound in amphibian life as it is a target of sexual selection and has therefore functioned as an isolating mechanism in anuran speciation. As the name suggests, *Rana temporaria*, or Common frog is the most widespread frog in Europe, but its distribution in the Northern Apennines is more and more disconnected as one moves southwards. We investigated the structure of reproductive calls in *Rana temporaria* from two populations along the Tosco-Romagnolo and Tosco-Emiliano Apennines to examine the possible identification of variability between groups as a base to consider them as different conservational units. Call duration, pulse number and dominant frequency of type A, B and C *sensu* Van Gelder et al. (1978) were measured and tested for statistically significant differences between the two populations; these variables were also compared to those of a population recorded in Devon, UK, taken from the British Sound Library. These first results showed a mosaic of variables significantly different among the three populations, allowing to hypothesize heterogeneity and lack of genetic contact between the populations on the mountain chain. For example in frogs from Romagna the Max frequency in A call was statistically different from the one of frogs from Tosco-Emiliano Apennines, but not from UK, as well as call A and C duration was different only from UK. Because of a high individual call variability and the preliminary results, further investigations at different levels of variability (within one individual, within a population and between populations) will be necessary to identify if these population can be considered as different conservation units.

## COMPARISON OF THE GENOTOXICITY STUDIES ON AMPHIBIANS

MÜGE GİDİŞ

Kütahya Dumlupınar University, Faculty of Arts and Science, Department of Biochemistry, Kutahya, Turkey

HİLAL ÇAVUŞ

Kütahya Dumlupınar University, Institute of Science Studies, Division of Biochemistry, Kutahya, Turkey

The pollutants that amphibians are exposed to due to environmental pollution cause vital defects. These defects can be; changes in the development stages, problems for reproduction and metabolism and damage of genetic material which affects the organism. Genotoxicity studies in amphibians are less compare to other vertebrates. Amphibians have an important role in food chain. The toxicity studies on amphibians will give us a clear view about the amphibian population dynamics and potential accumulation in human beings. A comparison analysis of the genotoxic studies on amphibians was performed mostly, micronuclei test, comet assay, bacterial ames test, jayten test (or newt micronucleus test) and freshwater pollution measure techniques. Data gained from these tests give us an important information about the biomonitoring of environment. Genotoxicity tests are quite difficult under the laboratory conditions and are performed both in vivo and intro. The aim of this study is to summarize the genetic toxicology studies of amphibians from past to present.

**BODY SIZE, AGE AT MATURITY AND LONGEVITY OF SEA TURTLES *Caretta caretta* STRANDED ALONG THE COASTS OF CAMPANIA (SOUTHERN ITALY)**

FABIO MARIA GUARINO

Dipartimento di Biologia, Università degli Studi di Napoli Federico II, Napoli, Italy

FABIO DI NOCERA

Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento di Sanità Animale, Portici (Naples), Italy

FRANCESCO POLLARO

Associazione Centro Studi Ecosistemi Mediterranei Pioppi (fraz. Pollica, Salerno), Italy

GIORGIO GALIERO

Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento di Sanità Animale, Portici (Naples), Italy

DORIANA IACCARINO

Istituto Zooprofilattico Sperimentale del Mezzogiorno, Dipartimento di Sanità Animale, Portici (Naples), Italy

DOMENICO IOVINO

Dipartimento di Biologia, Università degli Studi di Napoli Federico II, Napoli, Italy

MARCELLO MEZZASALMA

Dipartimento di Biologia, Università degli Studi di Napoli Federico II, Napoli, Italy

AGNESE PETRACCIOLI

Dipartimento di Biologia, Università degli Studi di Napoli Federico II, Napoli, Italy

NICOLA MAIO

Dipartimento di Biologia, Università degli Studi di Napoli Federico II, Napoli, Italy

We applied skeletochronology on phalangeal bone to estimate the age of dead-stranded specimens of sea turtle *Caretta caretta* along the coasts of Campania (Southern Italy) from 2013 to 2017. In addition, when available, gonads of both sexes were histologically examined to assess the individual reproductive status. In total we collected 66 turtles, with curved carapace length (CCL) ranging from 5.6 to 90.8 cm. Gonad samples were available for 26 stranded turtles, with CCL ranging from 25 to 79 cm, but only 10 (38.5%) of them was in a good conservation status to obtain maturity histological data. The estimated age of the *C. caretta* sampled individuals ranged from 0 (newborn, 5.6 cm CCL) to 31 years old and ca. 18.2%, 59.1%, and 22.7% of animals were between 0-9 years, 10-19 years and 20-31 years, respectively. The smallest sexually mature male, which had sperms in its seminiferous tubules, was 62.5 cm CCL and 16 years old. The smallest sexually mature female showing yolky follicles in its ovary, was 69 cm CCL and 15 years old. Our skeletochronological findings show that the loggerhead marine turtles stranded along the coast of Campania between 2013-2017 had an age comparable to that of individuals of similar CCL previously studied in other areas of the Mediterranean Sea. Moreover, the histological analysis supports the current knowledge on age-at-maturity estimates for the females of this species from Mediterranean population (not lower than 15 years) and indicate that males attain sexual maturity at a similar age.

**COLONIZATION OF CAVES INVOLVES PHENOTYPIC PLASTICITY OF THE METABOLIC LEVEL IN THE NEWT *Calotriton asper***

GUILLAUME OLIVIER

National Center for Scientific Research, Moulis, France

Theories on adaptation to cave life implicate that many characters have evolved in cave dwellers to cope the food frugality as for example a reduction of the basal metabolic rate. However, most of studies compared metabolic rates between closely related hypogean and epigeal species, or caves species to estimate the adaptation level. Due to the phylogenetic distance, such comparative studies are thus limited in terms of evolutionary conclusions. A rare case that could bring reliable evolutionary information regarding adaptation by organisms colonising the cave, is provided with *Calotriton asper* that lives both in caves and in the surface brooks from the Pyrenees. Here we report experiments which give evidence that a phenotypic plasticity is involved for a reduction of the metabolic rate in the surface-dwelling populations of *C. asper* acclimated to cave conditions. We suspected that this evolutionary mechanism which allows a quick response to environmental conditions, allowed *C. asper* to colonize the caves to escape global warming in low altitude while other populations have had to climb upstream to find the cool temperatures sought by this species.

**ACTIVITY DURING HIBERNATION PERIOD IN URBAN POPULATIONS OF THE  
FIRE SALAMANDER (*Salamandra salamandra*) IN PRAGUE.**

TOMÁŠ HOLER

Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Praha-Suchdol, 160 00, Czech  
Republic

JIRÍ VOJAR

Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Praha-Suchdol, 160 00, Czech  
Republic

PETR CHAJMA

Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Praha-Suchdol, 160 00, Czech  
Republic

DAVID LASTRA GONZÁLEZ

Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Praha-Suchdol, 160 00, Czech  
Republic

We have conducted a capture-mark-recapture surveys, to estimate the size of two populations of the fire salamander (*Salamandra salamandra*) inhabiting localities surrounded by urban areas in the city of Prague. The two localities are being surveyed regularly since 2015, approximately 10 times per year. Most of the surveys were done in the early spring, autumn and winter, always in the night. During the surveys, we walk a line transect through the locality, taking pictures of every salamander found. The photos of the dorsal pattern are later used for individual recognition and therefore there is no need of additional marking of individuals or any manipulation. During our research surveys, we have discovered, that the salamanders are active even during their natural hibernation period (XII, I, II). Whenever has the temperature risen at least slightly above zero and there was some precipitation, adult salamanders and even the juveniles were found active, exhibiting normal mobility. This specific behavior of fire salamanders has never been described before in the Czech Republic.

**WHEN TO EAT THE TOAD? EFFECT OF ABSOLUTE AND RELATIVE DENSITY ON SURVIVAL OF COMMON TOAD TADPOLES UNDER FISH PREDATION**

JAN M. KACZMAREK

Institute of Zoology, Poznań University of Life Sciences, Poznań, Poland

MIKOŁAJ KACZMARSKI

Institute of Zoology, Poznań University of Life Sciences, Poznań, Poland

JANUSZ KLOSKOWSKI

Institute of Zoology, Poznań University of Life Sciences, Poznań, Poland

Chemical defense and aposematism do not make prey completely immune to predation. The process by which predators learn about their prey's defenses depends on a number of factors, including the frequency of encounters with defended prey as well as alternative prey. We tested how the survival to metamorphosis of aposematic, chemically defended tadpoles of the common toad *Bufo bufo* was affected by their absolute (single-species treatment) and relative (mixed-species treatment with the common frog *Rana temporaria*) density in experimental mesocosms when they were subject to predation pressure from fish (common carp *Cyprinus carpio*) at two different hunger levels (with unlimited or limited availability of non-tadpole food). In single-species treatments, *B. bufo* survival was positively associated with its density. However, if *B. bufo* tadpoles were diluted among palatable heterospecifics, their survival decreased even though their absolute density remained constant. In both experiments, fewer *B. bufo* tadpoles survived when fish had limited access to alternative, non-tadpole food. Low density of defended prey, absolute or relative, may translate to slower predator learning about the prey's defenses, leading to more prey being killed. Although chemically defended, *B. bufo* tadpoles may still be subject to significant predation pressure from fish. Relative and absolute densities of chemically defended tadpoles, in potential interaction with the physiological state of the predators, may affect tadpole recruitment in habitats where fish are abundant.

**MICROPLASTIC INGESTION BY TADPOLES OF POND-BREEDING  
AMPHIBIANS – A PRELIMINARY REPORT**

KRZYSZTOF KOLENDA

Department of Evolutionary Biology and Conservation of Vertebrates, Institute of  
Environmental Biology, University of Wrocław, Wrocław, Poland

NATALIA KUŚMIEREK

Department of Parasitology, Institute of Genetics and Microbiology, Wrocław University,  
Wrocław, Poland

KATARZYNA PSTROWSKA

Division of Fuels Chemistry and Technology, Faculty of Chemistry, Wrocław University of Science and Technology,  
Wrocław, Poland

In recent years, marine environments have been well studied regarding microplastic (MP) pollution, however knowledge about freshwater biota contaminated by MPs remains insufficient. Several studies focused on fish and invertebrates in freshwaters, mainly concerned river ecosystems. In contrast, data on MPs in small waterbodies are scarce. In this study we aimed to check the occurrence and chemical composition of MPs in tadpoles of pond-breeding amphibians.

In total 141 tadpoles of 5 species (*Bufo bufo*, *Rana temporaria*, *Pelophylax esculentus* complex, *Pelobates fuscus*, and *Hyla arborea*) were collected by net from 7 ponds located in south-western Poland. Tadpoles were sacrificed in MS-222 and identified to the species level. Whole tadpoles' bodies were flooded with 30% hydrogen peroxide, incubated at 60 °C for 4 hours in a glass beaker, and afterwards MPs were searched using stereomicroscope. Polymer identification was analysed by ATR-IR spectroscopy. MPs were found in all studied sites. Of all, 39 (27,5%) tadpoles belonging to all caught species ingested a total of 45 MPs (mean 1.2, SD 0.4). All particles were fibres, with the mean length of 2 mm (SD 1.2) and diverse colours (transparent, blue, navy blue, black, brown, red, violet, pink). Spectroscopy analysis revealed that particles were of anthropogenic origin and included polyethylene, polypropylene, natural gum, and ABS.

To our knowledge this is a first report showing MPs ingestion by tadpoles that live in water bodies in Central Europe, however small number of particles per individual may suggest low impact on these organisms.



**IS PROBLEM SOLVING A PREREQUISITE TO BECOME A SUCCESSFUL INVADER?  
A BEHAVIORAL TEST WITH LIZARDS**

ANASTASIOS LIMNIOS

Department of Zoology - Marine Biology Faculty of Biology National and Kapodistrian University of Athens  
Panepistimiopolis 15701 Athens, Greece

CHLOÏ ADAMOPOULOU

Department of Zoology - Marine Biology Faculty of Biology National and Kapodistrian University of Athens  
Panepistimiopolis 15701 Athens, Greece

MIGUEL ANGEL CARRETERO

CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal

PANAGIOTIS PAFILIS

Department of Zoology - Marine Biology Faculty of Biology National and Kapodistrian University of Athens  
Panepistimiopolis 15701 Athens, Greece

Though invasive species constitute one of the biggest threats for global biodiversity, little is known on the putative advantages that make invasive species to outperform the equivalent native species in terms of behavior. The Italian wall lizard (*Podarcis siculus*) has been widely introduced and established viable populations in many countries including Greece. Our study aims to examine whether *P. siculus* displays enhanced exploratory behavior and abilities to solve novel challenges compared to two native congeneric species, an insular endemic (*Podarcis milensis*) and a widely distributed in both insular and mainland Greece (*Podarcis erhardii*). We performed two different experiments, a standard Y- maze arrangement and a test arena modified differently in each of three trials according to our scope. *Podarcis siculus* was more efficient than its congenics in finding and instantly consuming food. Our results suggest that *P. siculus* may display some behavioral traits that could provide better opportunities in surviving in the new environment and thus facilitate early stages of the introduction process.

**PREDATOR IDENTIFICATION FROM SALIVARY DNA LEFT ON ARTIFICIAL  
PREY**

DANIELA C. RÖBLER

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

STEFAN LÖTTERS

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

MICHÈLE FUGMANN

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

MICHAEL VEITH

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

HENRIK KREHENWINKEL

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

Predator-prey interactions are a vast field in ecological research and many mechanisms thereof are crucial to understand natural selection, trait functions, cognition of traits and hence their evolution. However, for many taxa predator-prey interactions are difficult to study because field observations of predation events are rare. Predation is based on perception of stimuli, in many cases visual cues. To understand the effects of different visual cues on predation, such as coloration or patterns of prey animals, many studies use artificial prey to collect information. Although widely deployed, this method largely lacks standardization of attack identification. In a new approach, we tested whether DNA of predators can be isolated from bite and peck marks found on clay models to ultimately allow precise and robust identification of the attacker. In a pilot study, we placed more than 800 models of European fire salamanders (*Salamandra salamandra*) in the field to collect information on predation. Attack marks on models were first analyzed visually. Subsequently, we successfully isolated and sequenced DNA of more than 6 different species of attackers from the marks left on clay models. Our results not only underline the problem of misidentification of attacks by vision alone, but also offer an intriguing method to gain robust data on predators of artificial prey. Furthermore, the method opens up new possibilities beyond the standard use of clay model studies to date, including a potential use in invasive species monitoring and species inventories.

**GEOGRAPHICAL VARIABILITY OF PHENOTYPIC TRAITS IN WESTERN WHIP SNAKE (*Hierophis viridiflavus*)**

NAHLA LUCCHINI

Alma Mater Studiorum – University of Bologna, BIGEA, Bologna, Italy

MARCO. A. L. ZUFFI

University of Pisa, Museum Natural History, Calci (Pisa), Italy

Animal populations living in different environmental condition usually exhibit divergent phenotypic characteristics. In fact, the external morphology of an organism is the result of the interaction between the organism itself and the environment in which it lives.

In snakes, body size and shape can vary largely among disjunct populations and the different traits can depend directly on environmental conditions. The Western European whip snake (*Hierophis viridiflavus*) is a medium-sized, non-venomous European colubrid which inhabits a great variety of environments and that is widely distributed throughout southwestern Europe, occurring also on most of the western Mediterranean islands. Because of its wide distribution and extreme abundance in his distribution range, it could be of great interest to test any eventual geographical variation of morphological features.

We focused our attention on snakes inhabiting Italy and Corsica, presenting divergent climatic condition (Oceanic vs Mediterranean); all the used samples came from zoological collection of different Italian museum. The analysis revealed significant variation in biometric and morphological head features in the European whip snake among the population inhabiting North, Centre and Sardinia-Corsica. Moreover, some differences were also found in the populations located in the continent, large islands and small islands while, considering the main habitat features as coast, lowland and mountain, no evidence of head shape differences were found.

**OCCURRENCE OF CORN SNAKE, *Pantherophis guttatus* (LINNAEUS 1766), IN LATIUM (ITALY): RECENT REPORTS OF A POTENTIALLY INVASIVE ALIEN SPECIES (SQUAMATA, SERPENTES, COLUBRIDAE)**

DANIELE MARINI

S.H.I. Sezione Lazio & ANVA – Associazione Naturalistica Valle dell’Aniene, Guidonia Montecelio (RM), Italy

VINCENZO FERRI

S.H.I. Sezione Lazio & L.Z.B.E., Dip. Biologia, Università di Roma 2 “Tor Vergata”, Rome, Italy

CHRISTIANA SOCCINI

S.H.I. Sezione Lazio & via Valverde 4, Tarquinia (VT), Italy

LORENZO DE LUCA

via Cianfroni 7, Mentana (RM), Italy

We present various recent reports of free-ranging *Pantherophis guttatus* (Linnaeus, 1766) in Latium (Central Italy). These findings raise many concerns since snakes are popular pets and since pet trade is the most important introduction pathway for allochthonous reptiles. Corn snake is one of the most bred and traded exotic ophidians and even recommended to neophyte terrarium lovers, but it is also subject of increasing numerous encounters of escaped or abandoned free-living individuals. Particularly, the current discovery of juveniles and adults in a fairly limited area at north of Rome highlights potential consequences of these introductions and the possible presence in Italy of naturalized populations of this species, already known for its extreme invasion risk.

**OVERSEAS INVASION AND REALIZED NICHE EVOLUTION OF THE  
MOURNING GECKO, *Lepidodactylus lugubris* (DUMERIL & BIBRON, 1836)**

DARIO NANIA

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

MORRIS FLECKS

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

DENNIS RÖDDER

Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

*Lepidodactylus lugubris* is a parthenogenetic gecko which has been increasingly expanding its range during the last century. This invasive species has been reported from multiple tropical and subtropical countries in five continents, most of which were colonized in recent times. In order to better understand the mechanisms behind this dramatic range expansion, we reconstructed the history of the invasion and investigated possible pathways used by the species to spread. Further, we built models of the realized niche of the species at different points in time during the invasion process. This was achieved through the implementation of modern hypervolumes construction methods, based on the Hutchinson's niche concept. The models were then compared to detect possible realized niche shifts over time. A progressive expansion of the realized niche was identified. As the species spread into new areas, we observed a tendency to colonize regions with warmer temperatures and higher precipitation rates. Finally, we found evidence for cargo shipping being the major pathway through which the species expands its range. Further studies on this topic should aim to investigate the role of biological interactions, and how they shape the distribution of *L. lugubris* on a global scale. A deeper understanding of this kind of processes will help us tackle the issue of invasive species, which has become a major challenge in conservation biology.

**AGE DETERMINATION AND SOME GROWTH PARAMETERS OF *Triturus anatolicus***

YAĞMUR ÖZYILMAZ

Pamukkale University, Faculty of Arts and Science Department of Biology,, Denizli, Turkey

EYUP BAŞKALE

Pamukkale University, Faculty of Arts and Science Department of Biology,, Denizli, Turkey

*Triturus anatolicus*, Anatolian Crested Newt was newly described species in Turkey, and is endemic to Anatolia. The distribution of the species started from the western Black Sea Region to beginning of the eastern Black Sea Region of Turkey. There is no data about population age structure of *T. anatolicus* as in many amphibian species. Skeletochronology is a reliable tool to assess population age structure. In this case, we used this approach to determine the age structure of Torkul population in this species. A total of 26 individuals were examined. All individuals exhibited Lines of Arrested Growth (LAGs) in the bone cross-sections, and the ages ranged from 3 to 12 years, with an average age of 6.3 years in males and 7.9 years in females. Sexual maturity is reached at an age of 3-4 years in both sexes. We also found a strong positive correlation between body sizes (SVL) and age structure, but the correlation coefficients between body sizes and age structure were very low and the body size ranges of different age classes overlapped.

**FROGONALITY: THE INTERPLAY BETWEEN ANIMAL PERSONALITY AND SEXUAL SELECTION IN POISON FROGS**

MÉLISSA PEIGNIER

Comparative Cognition, Messerli Research Institute, Vetmeduni Vienna, Medical University of Vienna, University of Vienna, Austria

SARAH CHALOUPKA

Department of Integrative Zoology, University of Vienna, Austria

KATHARINA DELLEFONT

Department of Integrative Zoology, University of Vienna, Austria

VIRGINIE CANOINE

Department of Behavioural Biology, University of Vienna, Austria

YIMEN ARAYA-AJOY

Center for Biodiversity Dynamics, Norwegian University of Science and Technology, Norway

DAN BLUMSTEIN

Department of Ecology and Evolutionary Biology, University of California, United States of America

EVA RINGLER

Department of Integrative Zoology, University of Vienna, Austria

Individuals do not usually exhibit the full range of behaviours of the behavioural repertoire observed in the entire populations or species and they differ in their level of plasticity. These differences between and within individuals may have considerable impact on an individual's role in a population, its prospects of survival and its mating success. As many behaviours occur in the context of sexual selection and are only expressed during social interactions, the interactions themselves impose selective pressures that may increase or decrease the amount of inter-individual variation. We propose to test the hypotheses that inter-individual variation in behaviour is maintained by a life-history trade-off between current and future reproduction. We propose that different behavioural phenotypes are linked to distinct life-history trade-offs; but that the long-term fitness of different behavioural phenotypes is equal. Our model species, *Allobates femoralis*, is a Neotropical frog with a prolonged breeding season, male territoriality, polygamous mating system and male parental care. During a three-year PhD project, we will collect data on this species behavioural ecology spending three months in French Guiana every year. One unique feature of this project is our ability to monitor, assay, and track an entire animal population in its natural habitat over several generations. During the first year, we measured personality traits such as exploration, aggressiveness and boldness, and assessed demographic parameters and in the next years we plan to measure individual survival and lifetime reproductive success. Eventually, this project will shed light on the mechanisms that maintain behavioural variation and help us to understand how consistent differences in behaviour can persist over evolutionary time.

**RIGHTING BEHAVIOUR IN THE EUROPEAN POND TURTLE (*Emys orbicularis*):  
RELATIONS BETWEEN LATERALIZATION AND MORPHOLOGY**

DANIELE PELLITTERI-ROSA

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, I-27100 Pavia, Italy

MARKO LAZIĆ

Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Adenauerallee 160, D-53113 Bonn, Deutschland

ANDREA GAZZOLA

Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, I-27100 Pavia, Italy

GIORGIO VALLORTIGARA

Center for Mind/Brain Sciences, University of Trento, P.zza Manifattura 1, I-38068 Rovereto (TN), Italy

Lateralization represents a key property of many behavioural traits, with the right and left sides of the brain providing different and integrative functions. Common ecological contexts where it can be observed are foraging and predatory ones, where both visual and auditory lateralization may provide advantages in faster response and increasing neural capacity. This is crucial in selecting a safe refuge during a predatory attack and may strongly affect the outcome of predator–prey interactions. A critical condition for turtles may occur when they are overturned on their own shell, which cause difficulties in breathing or in thermoregulation. Therefore, the ability to right is a critical adaptive component correlated to survival in aquatic turtles and has been observed to be lateralized.

An overlooked feature of behavioural lateralization is its relationship with external morphology. Here we investigated asymmetry in morphology and behavioural lateralization of freshwater European pond turtles *Emys orbicularis*. The goal of this study was to explore the possible relation between lateralization in righting behaviour response, and the symmetry bias in the shape of turtles (plastron and carapace).

We found that none of the morphometric variables was related to a lateralization index calculated on the first side from which turtles tried to right. However, a strong and negative correlation between the asymmetry index of plastron and direction from which they could turn emerged, with more symmetric animals tending to turn to the right side. Finally, righting performance (total time needed to completely turn) was clearly shell's size and shape dependent.



**DEMOGRAPHIC FLUCTUATIONS AND SHIFTS IN REPRODUCTIVE PHENOLOGY: A LONG-TERM STUDY (1994-2019) OF A COMMON TOAD (*Bufo bufo*) POPULATION AT LAKE ENDINE (NORTHERN ITALY)**

GIOVANNI GIOVINE

Stazione Sperimentale Regionale per lo Studio e la Conservazione degli Anfibi in Lombardia “Lago di Endine”, Comunità Montana della Valle Cavallina, Via Don Zinetti 1, I-24060 Casazza (BG), Italy

GIAMBATTISTA RIVELLINI

Stazione Sperimentale Regionale per lo Studio e la Conservazione degli Anfibi in Lombardia “Lago di Endine”, Comunità Montana della Valle Cavallina, Via Don Zinetti 1, I-24060 Casazza (BG), Italy

DANIELE PELLITTERI-ROSA

Dipartimento di Scienze della Terra e dell’Ambiente, Università degli Studi di Pavia, Via Ferrata 9, I-27100 Pavia, Italy  
Stazione Sperimentale Regionale per lo Studio e la Conservazione degli Anfibi in Lombardia “Lago di Endine”, Comunità Montana della Valle Cavallina, Via Don Zinetti 1, I-24060 Casazza (BG), Italy

Long-term studies on amphibian populations are not very common, due to sampling effort, stability of reproductive sites and costs related to monitoring projects. During the amphibian rescue campaigns coordinated by the “Stazione Sperimentale Regionale for study and conservation of amphibians in Lombardy” from 1994 to 2019 at Lake Endine (Northern Italy), data of many species are collected. Every year, between March and April, special barriers installed on the sides of the affected road allow the counting of individuals in transit to and from the reproductive site. An important batrachocenosis lives in this area, among which *Bufo bufo* stand out for its numerosness.

During the considered period, an average of 16198 individuals moving to the reproductive site were counted (min: 9048 in 2016; max: 27862 in 2003), with an average sex ratio (m:f) of 1.26. Breeding migrations are characterized by a strong numerical prevalence of individuals moving towards the lake compared to those returning to nourishment and wintering places, with an average of 14.8% (0.04% in 2001; 52.2% in 2007).

Over the years we observed strong demographic fluctuations. However, in the last two years there has been a sharp increase in toads that has brought the population back to good levels (25423 individuals). This trend coincided with the increase in the number of males, evidenced by the sex ratio of the last years. There has also been a gradual advance of the migration start date of about 10 days. We discuss this evidence as a possible consequence of climate change.

**THE DIET OF *Bufo bufo* POPULATIONS FROM CENTRAL PART OF THE BALKAN PENINSULA AND CHANGES IN THE DIET DURING ONTOGENY**

NATALIJA ČAĐENOVIĆ

The Natural History Museum of Montenegro, Trg Vojvode Bećir-bega Osmanagića 16, 81000 Podgorica, Montenegro.

SRĐAN STAMENKOVIĆ

University of Belgrade, faculty of Biology, Studentski trg 16, 11000 Beograd, Serbia.

LIDIJA POLOVIĆ

The Natural History Museum of Montenegro, Trg Vojvode Bećir-bega Osmanagića 16, 81000 Podgorica, Montenegro.

We analyzed the diet composition of *Bufo bufo* in the central part of Balkan Peninsula (Montenegro and Serbia) to define general diet preferences on southern and northern group of populations, by age categories and gender. We also addressed the potential correlation between Anurans body size by age category and prey size, and sex-based food niche partitioning in adults.

Analysis revealed that Common Toads feed most frequently on insects, e.g., Coleoptera (Carabidae, Curculionidae) and Hymenoptera (Formicidae), but the taxonomic and numerical value of prey varies at the level of groups, sexes and by age categories.

The results indicate that there is a positive correlation between the body size and the taxonomic and numerical value of the prey by age categories and gender in the populations of the southern group, while the statistical significance is lower in the populations of the northern group. Shannon-Winner diversity index and the dominance index in the population of the southern group is ( $H=1.19$ :  $d=0.48$ ), in the population of the northern group is ( $H=0.98$ :  $d=0.53$ ). Although the analysis indicates diverse nutrition of the *Bufo bufo* species, there is a certain degree of specialization in certain parts of the region, so this species couldn't be considered as a polyphagous predator.

The results showed that Pianka niche overlapping index between the southern and northern groups is very high 95% ( $Q_{jk} = 0.95$ ), between males as much as 94% ( $Q_{jk} = 0.95$ ) among females as much as 97% ( $Q_{jk} = 0.97$ ), indicating that both groups use the same resources or sources of food.

**EFFECTS OF EXPERIENCE AND SENSORY MODE ON PREDATORY  
BEHAVIOR OF *Iberolacerta cyreni* LIZARDS**

PABLO RECIO

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

GONZALO RODRÍGUEZ-RUIZ

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

JOSÉ MARTÍN

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

Predatory and feeding behavior of animals is affected by different factors, both exogenous and endogenous. Here, we study the role of vision, chemoreception, and experience in the predatory behaviour of the Iberian rock lizard (*Iberolacerta cyreni*). The experiment compared the feeding responses to visual cues, chemical cues, or a combination of both, in two scenarios of previous experience with the prey type. One of the prey (*Tenebrio molitor*) was always known previously by the lizards, while the other (*Blaptica dubia*) could be known or unknown. The results suggest that experience does not affect the predatory behavior of *I. cyreni*. However, the feeding responses vary between type of stimulus and prey, leading to conclude that predatory behavior seems to be influenced by dietary preferences in the first place, and, secondly, by the kind of stimulus received.

**GENETIC AND ENVIRONMENTAL FACTORS AFFECTING MELANINBASED  
COLORATION IN THE AMPHISBAENIAN *Trogonophis*  
*wiegmanni***

PABLO RECIO

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

GONZALO RODRÍGUEZ-RUIZ

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

LUIS V. GARCIA

Instituto de Recursos Naturales, CSIC, Sevilla, Spain

JOSÉ MARTÍN

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

Traditionally, melanin-based coloration was thought to occur only under genetic control. However, the literature is plenty of studies that suggest that melanin production may be also influenced by environmental factors. Thus, melanin traits may change along the life of organisms. We first examined the relationships between proportions of melanin, body size, sex and age in the amphisbaenian *Trogonophis wiegmanni* from different populations of Chafarinas Islands. The results show a positive correlation between age and proportions of melanin, obtained by analyzing ventral and dorsal photographs of amphisbaenians. We also examined whether the characteristics and degree of contamination of the soil where these amphisbaenians live affected melanin-based coloration. We specifically tested the hypothesis that melanin pigments might facilitate heavy metal detoxification by sequestering metal ions in the inner parts of the body and transporting them outside when animals shed their skin. Results show that although amphisbaenians from soils with higher concentrations of heavy metals also have more metals in their shed skins, their melanin coloration seem to be independent of soil characteristics.

## LANDSCAPE VARIABLES AND STRESS DRIVE HABITAT USE IN SAND DUNES *Podarcis siculus* GROUPS?

DINO SCARAVELLI

Department of Biological, Geological, and Environmental Sciences, University of Bologna, dino.scaravelli@unibo.it

The Italian wall lizard *Podarcis siculus* inhabits many different habitats along Italian peninsula, from coastal shores to high elevations in southern mountains. In order to assess the effect of the conservation and use of the few remaining dune environments on lizards' presence and population structure, transects were performed in the coastal ecosystems of Lido di Dante, Lido di Classe and Lido Adriano along the Adriatic sea coast of Ravenna province. In 15 sites during the month of September 2013 100 m transects were made observing the lizards. For each lizard the following parameters were collected by observation: age, sex, brightness classes of the findings, classes of shelter presence close or reached by the specimens, classes of attendance by the public of the area, time of the day, ground temperature. In all the transects, each with 2 repetitions, a total of 282 specimens of *P. siculus* were observed, divided as follows: 46 males, 54 females, 147 juveniles and 35 "undetermined". The different transects do not differ statistically in micro-environments classes found. Only 2 transects had significantly relative less presence of lizard correlate to the highest tourist attendance, as they were close to bathing facilities. Woody debris, preferred by both males and juveniles, are most appreciated shelters, followed by the plants of *Xanthium* sp., as well as the shrubs of *Tamarix gallica* and the scattered tufts of grasses, preferred by the young. There are significant positive correlations only in the relationship between presence of young and values of increasing intensity of shade and the intensity of number of shelters, meanwhile there are no significant findings for all the other variables. The local populations appear to be above all conditioned by the strong pressure generated in certain points by the anthropic use and by the state of conservation of the dune, sometimes strongly eroded, showing a high potential as environmental indicators.

**A NEW PERSPECTIVE FOR THE STUDY OF NEOTROPICAL ANURAN DIETS  
FROM BODY FRAGMENTS FOUND IN STOMACH CONTENTS**

BEATRIZ KELLEN TEIXEIRA FELIX

Programa de Pós-Graduação em Zoologia, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

MIRCO SOLÉ

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil and Herpetology Section,  
Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

The understanding on how anurans behave in relation to their feeding habits is the main focus of many researcher. Dietary studies are meant to describe which items are frequently ingested and how often they are found in stomachs. However, prey retrieved from stomachs has often already been fragmented by digestion. Thus, in 2001 Hirai and Matsui proposed formulas to estimate the total organism length from body fragments of insects. These formulas are applied only for length and do not apply to some orders as Blattodea, Mantodea and Odonata. In our study we created formulas to estimate length and width based on fragments of pre-digested prey, focusing on prey frequently found in the diet of Neotropical anurans. Formulas were elaborated through regressions using the body proportion between the found fragment (wing, elytra and head) and the original length or width, and validated through a Monte Carlo permutation test and compared with the already proposed formulas. A total of 1.717 insects were measured, resulting in 36 length formulas and 15 width formulas. We were able to conclude both the formulas (those proposed by Hirai and Matsui and by us) performed fine in order to describe the diet of Neotropical anurans based on insect fragments.

**MATE CHOICE SUGGESTS ASSORTATIVE MATING WITHIN A  
POPULATION OF *Lissotriton graecus* EXHIBITING FACULTATIVE  
PAEDOMORPHOSIS**

ELISAVET-ASPASIA TOLI

Molecular Ecology & Conservation Genetics Lab, Department of Biological Application & Technology, University  
of Ioannina, 45110 Ioannina, Greece

KONSTANTINOS SOTIROPOULOS

Molecular Ecology & Conservation Genetics Lab, Department of Biological Application & Technology, University  
of Ioannina, 45110 Ioannina, Greece

Alternative phenotypes coexisting within populations (i.e. facultative paedomorphosis), might reflect different life strategies and adaptations to spatially or temporally available resources. Assortative mating has been proposed as a mechanism promoting sexual isolation between individuals of different phenotypes. Here we study sexual performance and mate choice of Greek smooth newts exhibiting both morphs. 19 triads consisting of 1 male paedomorph, 1 male metamorph and 1 female (19 females: 6 metamorphs and 13 paedomorphs) were placed in tanks, and each triad was video recorded for 40min. From the videos we quantified the event of male display, the duration, the display latency (time in seconds from the entrance of the female to the first act of male display) and females' responsiveness to male display. No statistically significant difference in display, duration and latency between the male morphs was found. A significant interactive effect of female and male morph was found on display ( $p=0.04$ ). When female paedomorphs were present, 7 male metamorphs and 9 male paedomorphs engaged in courtship behavior, while when female metamorphs were present 4 male metamorphs and only 1 male paedomorph displayed. Female responsiveness was found to be correlated with the duration of display ( $p=0.01$ ) and not male morph. These results provide evidence that male newts are more likely to engage in courtship with females of the same morph, while females of both phenotypes are pairing with the male exhibiting longer display. This could limit the gene flow between the morphs in the study population and might contribute towards possible speciation events through assortative mating.

**AGE STRUCTURE IN *Podarcis muralis* POPULATIONS FROM DOBROGEA REGION, ROMANIA**

ALEXANDRA TELEA

Faculty of Agricultural and Natural Science, "Ovidius" University of Constanța, Romania

DRAGOȘ BĂLĂȘOIU

Faculty of Agricultural and Natural Science, "Ovidius" University of Constanța, Romania

DAN COGĂLNICEANU

Faculty of Agricultural and Natural Science, "Ovidius" University of Constanța, Romania

The wall lizard, *Podarcis muralis* is an European widespread, human-associated species, with successfully introduced populations in North America. Only a few studies have described its age-related population structure, that vary between 2-16 years while snout-vent length (SVL) can vary between 33-66mm in adults. Age related parameters have both population-specific characteristics and are influenced by external conditions like temperature, feeding patterns, predation, diseases and anthropic activities. In the Romanian Black Sea region of Dobrogea, the species is restricted to several isolated populations. We studied age and size related parameters from three of these populations (called populations B, C, D). Each captured animal was weighted, measured and a toe from the hind leg cut and stored in 70% alcohol. Age was assessed through skeletochronology. From 116 caught individuals, 45.69% were males, 45.69% females and 8.6% juveniles. Juveniles show no growth rings on bone section and have a SVL between 25-45 mm. For adults, the most common age cohort is 3-4 years old and maximum estimated age of 7 years, with SVL ranging between 44-69 mm. Males are generally larger and older than females: female SVL range is between 44-65 mm and age range between 2-5, while the SVL of males ranges between 45-69 mm and estimated age between 2-7 years old. Conversely, for population C we cannot make comparisons as we failed in catching a representative sample (only 3 individuals), adult individuals from populations B and D show significant differences ( $p < 0.05$ ), with B individuals being smaller and older than D population.



## THE ROLES OF ACCLIMATION AND BEHAVIOR IN BUFFERING CLIMATE CHANGE ALONG ELEVATIONAL GRADIENTS IN A EUROPEAN FROG

URTZI ENRIQUEZ-URZELAI

Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo UO.  
Catedrático Rodrigo Uría s/n, 33006 Oviedo. Spain

UMIB: Unidad Mixta de Investigación en Biodiversidad (UO-CSIC-PA). Campus de Mieres, Edificio de Investigación, Gonzalo Gutiérrez Quirós s/n, 33600 Mieres. Spain

REID TINGLEY

School of BioSciences, The University of Melbourne, Parkville, 3010, Victoria, Australia  
School of Biological Sciences, Monash University, Clayton, 3800, Victoria, Australia

MICHAEL R. KEARNEY

School of BioSciences, The University of Melbourne, Parkville, 3010, Victoria, Australia

MARTINA SACCO

Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo UO.

Catedrático Rodrigo Uría s/n, 33006 Oviedo. Spain

UMIB: Unidad Mixta de Investigación en Biodiversidad (UO-CSIC-PA). Campus de Mieres, Edificio de Investigación, Gonzalo Gutiérrez Quirós s/n, 33600 Mieres. Spain

ANTONIO S. PALACIO

Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo UO.

Catedrático Rodrigo Uría s/n, 33006 Oviedo. Spain

UMIB: Unidad Mixta de Investigación en Biodiversidad (UO-CSIC-PA). Campus de Mieres, Edificio de Investigación, Gonzalo Gutiérrez Quirós s/n, 33600 Mieres. Spain

MIGUEL TEJEDO

Department of Evolutionary Ecology, Estación Biológica de Doñana, CSIC, Avda. Américo Vespucio s/n, 41092 Sevilla, Spain

ALFREDO G. NICIEZA

Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo UO.

Catedrático Rodrigo Uría s/n, 33006 Oviedo. Spain

UMIB: Unidad Mixta de Investigación en Biodiversidad (UO-CSIC-PA). Campus de Mieres, Edificio de Investigación, Gonzalo Gutiérrez Quirós s/n, 33600 Mieres. Spain

Geographic phenotypic variation, acclimation, and behavioral thermoregulation jointly influence the vulnerability of species to climate change. The importance of interactions between these factors, however, remains poorly understood. We explored geographic variation in thermal tolerance and its potential for acclimation in juvenile *Rana temporaria* along elevational gradients. Further, we employed a mechanistic niche model (NicheMapR) to assess the relative contributions of phenotypic variation, acclimation, and thermoregulation in determining the impacts of climate change on thermal safety margins. Our analyses revealed that high elevation populations had slightly wider tolerance ranges driven by increases in heat tolerance but lower potential for acclimation. Biophysical models of thermal exposure indicated that observed phenotypic and plastic differences provide limited potential to protect juvenile frogs from changing climates. Indeed, the risk of reaching body temperatures beyond the species' thermal tolerance range was similar across elevations. In contrast, the ability to seek cooler retreat sites through behavioral adjustments played an essential role in buffering populations from thermal extremes predicted under climate change. Our results demonstrate that elevational variation in thermal tolerances and acclimation capacity are likely to be insufficient to buffer juvenile *R. temporaria* from predicted climate change; instead, behavioral thermoregulation will be the only effective mechanism for juvenile *R. temporaria* to avoid thermal stress under future climates.

## DOES FORAGING AREA LIMITATION AFFECT FIRE SALAMANDER LARVAE GROWING? A PRELIMINARY STUDY

LODOVICA VINCI

Dipartimento di Bioscienze, Università degli Studi di Milano, Via Celoria, 26, 20133 Milano, Italy

RAOUL MANENTI

Dipartimento di Bioscienze, Università degli Studi di Milano, Via Celoria, 26, 20133 Milano, Italy

GENTILE FRANCESCO FICETOLA

Dipartimento di Scienze dell'Ambiente e del Territorio e di Scienze della Terra, Università degli Studi di Milano-Bicocca,  
Piazza della Scienza 1, 20126 Milano, Italy

Laboratoire d'Ecologie Alpine (LECA), Université Grenoble Alpes, F-38000 Grenoble, France

CNRS, LECA, F-38000 Grenoble, France

BIANCA LOMBARDI

Dipartimento di Bioscienze, Università degli Studi di Milano, Via Celoria, 26, 20133 Milano, Italy

LUCA BAGLIONI

Dipartimento di Bioscienze, Università degli Studi di Milano, Via Celoria, 26, 20133 Milano, Italy

Different laboratory studies showed a substantial difference between the foraging strategies of salamanders from cave and surface habitats. Aquatic cave salamanders species show a more wide active foraging strategy, in order to increase prey encounter rate and detection. Surface salamander species prefer the ambush strategy. Similar differences occur fire salamander larvae (*Salamandra salamandra*) belonging from cave and stream populations, when tested in laboratory.

The aim of this preliminary field study is to see if narrowing their hunting area, fire salamander larvae from cave and streams are still able to grow.

We collected 144 larvae from cave and streams, and using a cross environment approach we reared them in cages (diameter 40 cm) placed in three caves and three streams (total number of cages: 36; four larvae were placed in each cage). We monitored larvae growth every ten days measuring their length and weight. In four of the rearing sites we measured also the growth of larvae that naturally occurred outside the cages.

Larvae in caves grow slightly less than those in streams; both stream and cave larvae when in cages grow less than the larvae outside cages, however while larvae placed in stream cages regularly grow (daily average growth: 0,082 mm), larvae placed in cave cages are not able to develop (daily average growth: -0,018 mm).

Our results demonstrate that wild foraging activity is vital for fire salamander larvae growth in caves and provide insights for understanding behavioural patterns in cave-adapted urodeles.

## IS THE GROWTH OF BROWN FROG'S TADPOLES INFLUENCED BY THE PRESENCE OF RED-EARED SLIDERS?

MAGDA VODRÁŽKOVÁ

University of South Bohemia in České Budějovice, Czech Republic

MICHAL BEREČ

University of South Bohemia in České Budějovice, Czech Republic

An increasing amount of attention is devoted to studying the impact of non-native animal species on native biota. Biological invasion is considered as one of the main causes of the decrease in biodiversity all over the world. Because of their competitive abilities and omnivorous diet, non-native red-eared sliders *Trachemys scripta* have the potential to disrupt aquatic ecosystems in Central European.

In order to detect possible predation events tadpoles often rely on chemical stimuli, as can be predator's secretions. In aquatic ecosystems, chemical cues are a major source of information through which animals are able to assess the current state of their environment and gain information about local predation risk. Previous studies showed that tadpoles can significantly reduce swim and follow less direct trajectories when exposed to chemical cues released by turtle predators. This study aims to evaluate the influence of these anti-predator behaviours on tadpole development in *Rana temporaria*. We hypothesized a behavioral response to the predator would lead to various effects on larval growth and development, affecting both larval stage duration and body size after metamorphosis. Tadpoles in the permanent presence of turtle grew faster, metamorphosed earlier and attained smaller size at metamorphosis. Mentioned reactions may affect the survival and fitness of a metamorphosed individual.

The study was supported by a project of the Grant Agency of the University of South Bohemia in České Budějovice 045/2019/Z.

***Bombina variegata* IN LOMBARDY: GENETIC IMPLICATION FOR  
CONSERVATION**

ANNA RITA DI CERBO,

Centro Studi Fauna Vertebrata "Luigi Cagnolaro", Società Italiana di Scienze Naturali c/o Museo Civico di Storia Naturale di  
Milano, Milan, Italy

CRISTIANO VERNESI,

Department of Sustainable Agroecosystems and Bioresources, Research and Innovation Centre- Fondazione Edmund Mach,  
S. Michele all'Adige (TN), Italy

GIOVANNI GIOVINE,

Stazione Sperimentale Regionale per lo studio e la conservazione degli anfibi in Lombardia - Lago di Endine LR 86/83,  
Casazza (BG), Italy

GIAMBATTISTA RIVELLINI,

Stazione Sperimentale Regionale per lo studio e la conservazione degli anfibi in Lombardia - Lago di Endine LR 86/83,  
Casazza (BG), Italy

VINCENZO FERRI,

L.Z.B.E., Department of Biology, University of Rome II "Tor Vergata", Rome, Italy

ELISABETTA ROSSI

Regione Lombardia, DG Ambiente e clima, Struttura natura e biodiversità, Milan, Italy

ANDREA AGAPITO LUDOVICI

WWF Italia, Roma, Italy

The study was carried out within the Life GESTIRE 2020 action A14: Plan on priority interventions for significant herpetofauna in Lombardy based on a survey on the status of populations and on the analysis of threats. The genetic screening of *Bombina variegata* has been performed for complementing demographic census data in Lombardy, which is the western limit of species range in Italy. In this area a limited and fragmented distribution with isolated populations and of small size has been found. We collected buccal samples from 29 sites located in Lecco, Bergamo and Brescia provinces. We typed 102 individuals, using mtDNA cytochrome b and 11 nuclear microsatellites and investigated fine-scale population structure. We identified four genetically distinct groups (Pop1-4). The results show that i) the easternmost group (Pop4) is highly differentiated from the others; ii) all populations show a very low genetic variability (both in terms of heterozygosity and mean number of alleles), that is extremely low in the easternmost group; iii) all populations are different from the previously studied populations from Trentino. The critical demographic status associated with low genetic diversity within populations might result in increasing of genetic drift and inbreeding, that can ultimately determine local and even regional extinction. Efforts should be orientated to specific conservation actions such as ecological site improvement and population restocking .

**PHYLOGEOGRAPHY OF GROOVE-CROWNED BULLFROG (*Hoplobatrachus occipitalis*) IN MAURITANIA PROVIDES ANOTHER PIECE TO THE DIPLOID-TETRAPLOID FROG PUZZLE?**

DUARTE V. GONÇALVES  
CIBIO/InBIO – U. Porto, Porto, Portugal  
IBE (CSIC-UPF), Barcelona, Spain  
CIIMAR-U.Porto, Porto, Portugal  
JOSÉ C. BRITO  
CIBIO/InBIO – U. Porto, Porto, Portugal

Several studies have assessed the phylogeographic patterns of vertebrates in North Africa and Sahara-Sahel, but most of the phylogeographic knowledge on amphibians comes from the Mediterranean region while the southern Sahara and Sahel remains poorly studied. Here we assess the phylogeography of the African Groove-crowned frog *Hoplobatrachus occipitalis*, with a focus on western Sahel in order to better understand the biogeographic patterns of semi-aquatic species in this arid region. Using mitochondrial and nuclear markers, we have assessed the species' genetic structure, distribution of genetic diversity, and the presence of cryptic diversity. We found evidence of a recent (re-)colonization of the mountains in its northernmost distribution, but also for the role of southern Mauritanian mountains and large rivers as refugia. Two major lineages were detected, one perhaps endemic to Mauritania and the other widespread in Africa. The first lineage possibly constitutes the second Sahelian amphibian endemic; the latter may have originated through an allopolyploidy event, with the Mauritanian lineage being one of the parental ones.

**FEMALE REPRODUCTIVE CYCLE OF KULZER'S ROCK LIZARD  
(*Phoenicolacerta kulzeri*, MÜLLER & WETTSTEIN, 1932), FROM LEBANON**

FIDA NASSAR

Laboratory of Georesources, Geosciences and Environment - Developmental Biology team, Faculty of Sciences, Fanar campus, Lebanese University, Lebanon

SOUAD HRAOUI-BLOQUET

Faculty of Sciences, Fanar campus, Lebanese University, Lebanon

We studied the female reproductive cycle of the 57 oviparous *Phoenicolacerta kulzeri* lizards collected between April 2000 and September 2001 in Mount Sannine 2000 m a.s.l., Lebanon and deposited at the Natural History Museum of the Lebanese University. Females collected in spring showed vitellogenesis in their ovaries. Oviposition (enlarged follicles and/or oviductal eggs) occurred in May and June. Females produced up to two clutches. Mean clutch size was  $3.92 \pm 1.74$  SD, range 2-8 eggs. We found significant correlation between clutch size and female body size. Females of *P. kulzeri* reached sexual maturity at 46 mm snout-vent length. The mean SVL was  $55.07 \text{ mm} \pm 4.33$  SD (range = 46 - 65 mm) for adult females (n=53). We found no significant difference in body size between female and male adult *P. kulzeri* specimens. All females examined from July to October were quiescent. Interestingly, seasonal variations in female reproductive activity were not synchronized throughout all the reproductive period with those of males since males exhibited an autumnal spermatogenesis in addition to the vernal spermiogenesis.

**MACROEVOLUTIONARY VARIATION AND ENVIRONMENTAL CORRELATES  
OF SCALATION TRAITS IN EURASIAN VIPERS**

ANTIGONI KALIONTZOPOULOU

CIBIO/InBIO, Vairão, Portugal

ALBA MARTÍNEZ

CSIC-UIMP, Madrid, Spain

INÊS FREITAS

CIBIO/InBIO, Vairão, Portugal

FERNANDO MARTÍNEZ-FREIRÍA

CIBIO/InBIO, Vairão, Portugal

Scalation traits have been traditionally used for systematic inferences and species diagnosis in many reptile taxa, and as such they are expected to be associated with species' phylogenetic relationships. However, these traits are also known to be under strong environmental influences, both plastic and adaptive. Varying conditions throughout development is known to frequently cause plastic variation in scalation, while some scalation traits are also essential in interacting with local environments, through thermoregulation, water balance, or habitat use. Here, we evaluated the relative contribution of evolutionary history and environmental factors in shaping scalation across 40 lineages of Eurasian vipers. We described macroevolutionary variation across lineages and tested for the strength of phylogenetic signal. Then, we tested how macroevolutionary patterns of scalation are shaped by key environmental factors. We found considerable variation in the number of crown, loreal, ventral, subcaudal and dorsal scales. All traits exhibited significant phylogenetic signal, with values generally close to one, suggesting that divergence among species in most traits reflects their phylogenetic relatedness. However, the number of ventral scales exhibited lower values of phylogenetic signal, pointing to at least some adaptive influence. Phylogenetic regressions corroborated this hypothesis, as they indicated a significant contribution of environmental variables, where species inhabiting areas with higher solar radiation and temperatures tended to exhibit a lower number of ventral scales. While variation across species in most scalation traits reflect their phylogenetic relatedness, the number of ventral scales is potentially under a strong environmental influence, with potentially high evolutionary lability depending on ecological conditions.

**SEXUAL DIMORPHISM IN DIGITS LENGTH AND THEIR RATIOS IN  
SALAMANDER SPECIES (SALAMANDRIDAE)**

ANA KIJANOVIĆ

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

TANJA VUKOV

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

NAZAN UZUM

Department of Biology, Faculty of Arts and Sciences Adnan Menderes University, Aydin, Turkey

KURTULUS OLGUN

Department of Biology, Faculty of Arts and Sciences Adnan Menderes University, Aydin, Turkey

NATAŠA TOMAŠEVIĆ KOLAROV

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

In recent decades, digit ratio has attracted considerable attention in biology. It has been suggested that the digit ratio may be used as a biomarker of early developmental effects. In particular, the second-to-fourth digit ratio has been linked to the effects of sex hormones and their receptor genes. Also, this ratio is a morphological feature that is important for tetrapod locomotory performances in different microhabitats. Here, we focus on examining sex differences in digit ratios (2D:3D, 2D: 4D and 3D: 4D) in 15 species of the family Salamandridae with different patterns of aquatic to terrestrial locomotion. The main questions of this study are: (1) whether sexual dimorphism exists in digit length and their ratios (2) whether size, phylogeny or ecology has an influence on digit length and its ratios. We assume that different selective pressures related to aquatic and terrestrial locomotion could override the prenatal developmental cues. The results suggest a different degree of dimorphism in analyzed digits and digits ratios in analyzed salamanders, with strong effects of ecology, i.e. the proportion of time spent using each locomotion type, swimming or walking, implying that function has a large impact on limb growth. The results of our study are in line with others which can help to evaluate whether the developmental or functional processes have the most impact on limb evolution.



**FIRST KARYOLOGICAL REPORT ON THE ENDEMIC MALAGASY PHANTOM  
GECKO (*Matoatoa brevipes*)**

MARCELLO MEZZASALMA

Department of Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD, UK

FABIO M. GUARINO

Dipartimento di Biologia, Università di Napoli Federico II, Via Cinthia 26, 80126 Napoli, Italy

SIMON LOADER

Department of Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD, UK

GAETANO ODIERNA

Dipartimento di Biologia, Università di Napoli Federico II, Via Cinthia 26, 80126 Napoli, Italy

JEFFREY W. STREICHER

Department of Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD, UK

NATALIE COOPER

Department of Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD, UK

Chromosomal data are currently available for few Malagasy reptiles, leaving their karyological evolution largely unexplored, but available evidence suggests high levels of chromosomal variability in different taxa.

Here we present the results of the first karyological study on the endemic Malagasy phantom gecko *Matoatoa brevipes*. We performed a combination of traditional staining and banding techniques (sequential C-banding + Giemsa + CMA3 + DAPI) and a phylogenetic analysis using a fragment (of about 600 bp) of the mitochondrial gene NADH dehydrogenase subunit 2 (ND2), to provide more confident identification of the samples studied and to evaluate genetic distances among different populations.

The cytogenetic analysis showed that the karyotype of *M. brevipes* is of  $2n = 34$  chromosomes, with one metacentric and sixteen telocentric pairs, gradually decreasing in length and without any clear distinction between macro- and microchromosomes. No heteromorphic chromosomes were detected in either sex. C-banding revealed a scarce heterochromatin content without any largely heterochromatic chromosomes, suggesting that putative sex chromosomes are at an early stage of differentiation. Heterochromatin clusters were mainly evident with C-banding + CMA3 and C-banding + Giemsa at centromeric and telomeric regions, but barely positive to DAPI. The newly generated ND2 sequences showed significant haplotype diversity (about 5% uncorrected *p*-distance) between populations from different areas of the species distribution.

In the light of the available chromosome data for closely related Malagasy and non-Malagasy geckos, we also advance a hypothesis on their karyotype evolution concerning the augmentation/reduction of the chromosome number involving alternative events of chromosome fusions/fissions.

**INFERRING THE ORIGIN AND PATHWAY OF THE ALLOCHTHONOUS POPULATIONS OF COMMON WALL LIZARDS, *Podarcis muralis*, IN UKRAINE**

OLEKSANDRA OSKYRKO

ESC «Institute of biology and medicine», Taras Shevchenko national university of Kyiv, prosp. Akademika Hlushkova 2, Kyiv, Ukraine;

CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus de Vairão. 4485-661 Vairão, Portugal

HANNA LAAKKONEN

Department of Biology, Lund University, Sölvegatan 37, 223 62 Lund, Sweden

IOLANDA SILVA-ROCHA

CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus de Vairão. 4485-661 Vairão, Portugal

TOBIAS ULLER

Department of Biology, Lund University, Sölvegatan 37, 223 62 Lund, Sweden

MIGUEL A. CARRETERO

CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus de Vairão. 4485-661 Vairão, Portugal

OLEKSIY MARUSHCHAK

Schmalhausen Institute of Zoology, NAS of Ukraine, vul. B. Khmelnytskogo, 15, Kyiv, Ukraine

Introduction of species is leading to significant changes in the biodiversity worldwide. One example is the repeated introduction of the sub-Mediterranean common wall lizard *Podarcis muralis* (Laurenti, 1768) outside of their natural range. Molecular evidence taking advantage of the structured phylogeography of the species in its native range suggests multiple and eventually mixed origins of introduced populations. Native Central-European populations show low haplotype diversity of mitochondrial DNA (mtDNA). The species attains the northeastern limit of its native range in Romania, occurring primarily along the Carpathian Mountains and in several sites in the Danube river valley and city of Dobruja. In Ukraine this species was found for the first time in 2012 in the southern part of Odessa region (city of Reni). We generated sequences of the mtDNA gene cytochrome-b of samples from this population and analyzed them together with published sequences from across the species' native range to determine its origin and likely introduction pathway. Phylogenetic analysis showed that the Odessa lizards originate from two distinct lineages, both occurring within the formerly called "Central European clade", which includes most of the lizards from Eastern Europe. This suggests at least two separate origins from different locations. One of the haplotypes in the non-native population is very similar to haplotypes found in Romania. The other haplotype is apparently from a mitochondrial lineage present across a large geographical range covering regions of Bulgaria, Serbia, and Romania. These results further demonstrate that common wall lizards are able to establish outside of its native range and that repeated human transport is promoting lineage admixture.

**POPULATION GENETICS OF *Bufo bufo* SPECIES IN TURKEY**

NURHAYAT ÖZDEMİR

Recep Tayyip Erdogan University, Rize, Turkey

CANTEKİN DURSUN

Recep Tayyip Erdogan University, Rize, Turkey

SERKAN GÜL

Recep Tayyip Erdogan University, Rize, Turkey

NAZAN ÜZÜM

Aydın Adnan Menderes University, Aydın, Turkey

BİLAL KUTRUP

Karadeniz Technical University, Trabzon, Turkey

*Bufo bufo* (Linnaeus, 1758) is a member of *Bufo bufo* species group distributed in the Western Palearctic from most of Europe including northern and eastern France into Russia (including toads from Great Britain, Scandinavia, Italy, the Balkans and the larger part of Turkey). In this study, population genetic structure of *B. bufo* in Turkey was handled. Thus, 60 individuals were sampled from 20 different provinces from Turkey. A portion of 544 bp of the mitochondrial 16 rRNA gene and 786 bp of the mitochondrial *cyt-b* genes were amplified. Purification of PCR products and sequencing was performed by Macrogen, Inc. By using the DnaSP version 6 software, haplotype diversity was calculated, haplotypes were determined for each gene and haplotype networks were created with PopART 1.7 software. Using the Arlequin version 3.5.1.3 software, the neutrality test and mismatch calculations for each gene were examined. As a result of the analysis of 16S and cytochrome *b* genes according to the Tajima D test, *B. bufo* populations showed a low variation ( $P > 0.05$ ). In addition, negativity indicates an increase in *B. bufo* populations. According to Fu's  $F_s$  test, the cytochrome *b* gene shows the growth of *B. bufo* populations, and to be in the same gene pool ( $P < 0.05$ ). According to the mismatch test, there was an expansion of *B. bufo* populations in terms of 16S gene ( $P > 0.05$ ). The 16S and cytochrome *b* genes point out a uniform distribution of unimodal (single peak) for *B. bufo* populations. In other words they indicate a rapid population growth from a single haplotype for *B. bufo* populations.

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**CONNECTING SPATIAL TROPHIC ECOLOGY WITH PROCESSES OF  
POPULATION DIFFERENTIATION AND SPECIATION IN GALÁPAGOS MARINE  
IGUANAS (*Amblyrhynchus cristatus*)**

TIMM REINHARDT

University of Leipzig/ Federal Agency for Nature Conservation, Leipzig/Bonn, Germany,

STEN ANSLAN

Technische Universität Braunschweig, Braunschweig, Germany

AMY MACLEOD

University of Leipzig, Leipzig, Germany

JUAN M. GUAYASAMIN

Colegio de Ciencias Biológicas y Ambientales (COCIBA), Universidad San Francisco de Quito, Ecuador;

DIEGO PÁEZ-ROSAS

Colegio de Ciencias Biológicas y Ambientales (COCIBA), Universidad San Francisco de Quito, Ecuador;

NICOLÁS PEÑAFIEL

Colegio de Ciencias Biológicas y Ambientales (COCIBA), Universidad San Francisco de Quito, Ecuador

SEBASTIAN STEINFARTZ

University of Leipzig, Leipzig, Germany

Marine iguanas (*Amblyrhynchus cristatus*) are the only seagoing lizard worldwide. They graze specifically on green and red macro-algae in the tidal zone. San Cristóbal is the easternmost and oldest island of the Galápagos archipelago and is inhabited by two genetically differentiated marine iguana populations that were recently described as distinct subspecies (*Amblyrhynchus c. mertensi* and *A. c. godzilla*). Previous investigations have shown that intermixing between the two subspecies is rare; this contrasts with the relatively high frequency of hybridization of these subspecies with individuals from adjacent islands. Here, we provide the first data that shows differences in feeding ecology between the two subspecies. To this end, we analyzed food composition of adult marine iguanas of both subspecies using a DNA-metabarcoding approach, which aimed to identify consumed algal species from feces samples and signatures of stable isotopes in accumulated skin tissue. We find differences in the composition but not in the number of consumed algal species between sites (subspecies). Further, the variability in the trophic niche between iguana populations is low and is predicted by the site specific macro-algal community composition. At the same time, isotopic values of iguana tissue also indicates a very small range of food items throughout the iguana life cycle, and indicate only very restricted spatial expansion. Therefore, we suggest that small home ranges and low mobility might be relevant factors in the separation between the two subspecies on the island scale.

**THE TOAD FLY (*Lucilia bufonivora*): ITS EVOLUTIONARY STATUS  
AND MOLECULAR IDENTIFICATION**

GERARDO ARIAS-ROBLEDO

University of Bristol, University of Exeter, UK

TARIQ STARK

RAVON, Nijmegen, the Netherlands

RICHARD L. WALL

University of Bristol, UK

JAMIE R. STEVENS

University of Exeter, UK

The blow fly genus *Lucilia* is composed largely of saprophages and facultative myiasis agents, including the economically important species *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) and *Lucilia sericata* (Meigen). Only one species is generally recognized as an obligate agent of myiasis, *Lucilia bufonivora* (Moniez), and this is an obligate parasite of toads. *Lucilia silvarum* (Meigen), a sister species, behaves mainly as a carrion breeder; however, it has also been reported as a facultative parasite of amphibians. Morphologically, these species are almost identical, and historically this has led to misidentification, taxonomic ambiguity and a paucity of studies of *L. bufonivora*. In this study, dipterous larvae were analysed from toad myiasis cases from the U.K., The Netherlands and Switzerland, together with adult specimens of fly species implicated in amphibian parasitism: *L. bufonivora*, *L. silvarum* and *Lucilia elongata* (Shannon) (from North America). Partial sequences of two genes, *cox1* and *e1 $\alpha$* , were amplified. Seven additional blow fly species were analysed as outgroups. Bayesian inference trees of *cox1*, *e1 $\alpha$*  and a combined-gene dataset were constructed. All larvae isolated from toads were identified as *L. bufonivora* and no specimens of *L. silvarum* were implicated in amphibian myiasis. This study confirms *L. silvarum* and *L. bufonivora* as distinct sister species and provides unambiguous molecular identification of *L. bufonivora*.

**THE REPTILE DATABASE AND THE FUTURE OF TAXONOMIC DATA**

PETER UETZ

Center for Biological Data Science, Virginia Commonwealth University, Richmond, VA, USA

SAMI CHERIKH

Center for Biological Data Science, Virginia Commonwealth University, Richmond, VA, USA

MAX HAMMERMANN

Karlsruhe University of Applied Sciences, Karlsruhe, Germany

The Reptile Database (<http://www.reptile-database.org>) was founded in 1996 as EMBL Reptile Database and has evolved into a major online resource for systematic herpetology. For a brief history see Uetz 2016, *Herp. Rev.* 47 (2): 330. Currently (May 2019) the database lists 10,969 species and 2,325 subspecies, although these numbers change almost daily. All species have distribution information and their original citations, with a total of 48,097 literature records of which 34,552 were linked to web sites where they can be obtained (many at the Biodiversity Heritage Library and other public sites). At the time of this writing, 3,937 species had diagnoses, 5,277 had etymologies, 10,764 had type localities (many with coordinates that are mapped to Google maps online), and 10,942 had type information with all known primary types located in 369 museums worldwide. Currently the database receives ~ 50,000 visits and ~240,000 page views a month. This spring we exceeded 13,000 user-submitted photos representing 4,500 species. We also expect to make available maps to most reptile species worldwide this year. The database requests research collaborations, e.g. macro-ecology, species identification, or large-scale phylogenetics projects. The Reptile Database actively integrates and exchanges data with providers such as NCBI Taxonomy (Genbank), iNaturalist, IUCN Redlist, Catalogue of Life (CoL) and others. An important challenge is the dynamic nature of all of these datasources, hence a future challenge is to automate data exchange but also published analyses in dynamic “live” papers.

**CONSERVATION GENETICS OF A THREATENED AMPHIBIAN (*Bombina variegata*)  
IN NORTHERN RHINELAND-PALATINATE, GERMANY**

ALENA MARCELLA HANTZSCHMANN

Institute for Integrated Natural Sciences, University of Koblenz-Landau, 56070 Koblenz, Germany

HEIKE PRÖHL

Institute of Zoology, University of Veterinary Medicine, 30559 Hannover, Germany

ULRICH SINSCH

Institute for Integrated Natural Sciences, University of Koblenz-Landau, 56070 Koblenz, Germany

In Germany, the yellow-bellied toad, *Bombina variegata*, is threatened and strictly protected. We studied the genetic population structure and diversity of this amphibian species inhabiting a low mountain range, the Westerwald in northern Rhineland-Palatinate. This is one of the northernmost regions where *Bombina variegata* is widely distributed, thus serving as an important reservoir for (re)colonisation. Population connectivity and genetic diversity of 200 specimens belonging to seven sites (200–450 m a.s.l.) were investigated using ten molecular markers (microsatellites). The analysis of genetic sub-structuring identified at least two differentiated groups of populations inhabiting the southern and northern region of the Westerwald. We found low overall genetic diversity, but partially large genetic differences among populations. Fragmentation of populations inhabiting nearby locations (< 500 m distance in between) indicates a low dispersal capacity. Thus, conservation measures should focus on the connectivity of neighbouring populations through stepping stone ponds. Further analyses will give more precise insights concerning genetic relatedness to nearby populations in western Hesse.

**PROTOCOL OPTIMIZATION FOR FECAL DNA COLLECTION ON TERRAPINS**

JOANA VERÍSSIMO

CIBIO/InBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, 4485-661 Vairão, Portugal; Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

VANESSA MATA

CIBIO/InBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, 4485-661 Vairão, Portugal; Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

SIMON JARMAN

School of Biological Sciences, University of Western Australia, Perth, WA, Australia; Environomics Future Science Platform, Indian Oceans Marine Research Centre, Commonwealth Scientific and Industrial Research Organization, Crawley, Western Australia, Australia

PEDRO BEJA

CIBIO/InBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, 4485-661 Vairão, Portugal CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

During recent years, metabarcoding studies of fecal samples have been successfully used to assess species diets to better understand food webs dynamics, which is a valuable tool when inferring community ecology and ecosystem functions. Terrapins fecal material is commonly found in water. However, to our knowledge, no metabarcoding studies exist to assess terrapin species diets. Morphological identification of prey has been the preferred methodology and hence, sample collection methods need to be optimized to ensure DNA quality. Here, we used *Trachemys scripta* to test a combination of sample collection methods based on environmental DNA (eDNA) approaches (water filtration vs. ethanol precipitation) and extraction methods (column- vs. magnetic beads-based approach) that could provide us with the best tool for fecal DNA recovery. Moreover, through a controlled 7-day feeding trial we will be able to assess the persistence of *Tenebrio molitor* in the digestive system of the species. We obtained a total of 270 eDNA samples, from which DNA was amplified using two primer sets designed to identify invertebrate and plant species and were sequenced on an Illumina platform. This study will allow us to choose the best combination of sampling methodology and DNA extraction for fecal collection across terrapin species. Furthermore, we expect to be able to make inferences regarding the time of detection for food items in wild specimens.



**LOST GENERATION – THE OVERLOOKED SCIENTIFIC VALUE OF TADPOLES IN MUSEUM COLLECTIONS**

JÖRN LAUDOR

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

ARNE SCHULZE

Zoologische Gesellschaft für Arten- u. Populationsschutz (ZGAP), Hindenburgstraße 12, 76829 Landau in der Pfalz, Germany

JÖRN KÖHLER

Hessisches Landesmuseum Darmstadt (HLMD), Friedensplatz 1, 64283 Darmstadt, Germany

STEFAN LÖTTERS

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

BRUNO VIERTEL

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

MICHAEL VEITH

Universität Trier, Abt. Biogeographie, Universitätsring 15, 54296 Trier, Germany

Anuran larvae exhibit a wide diversity of morphological features. However, research on frogs and toads primary aims at adult stages, so that information on their tadpoles is often scarce. In scientific museum collections, tadpoles are often barely processed. They remain the ‘lost generation’ and their scientific value is overlooked. The aim of this ongoing study is to contribute to the identification of phylogenetic and geographic patterns in tadpole morphology, using larval specimens collected in different regions of the globe and housed in museum collections. To achieve these aims, ‘classical’ museum collection work and modern morphological, molecular, statistical and GIS methodology are combined. Retrieving the available data from museums, combined with available phylogenetic and ecological data, will contribute to the understanding of tadpole morphology.

## **A GIS APPROACH TO THE STUDY OF COLOUR ANOMALIES IN AMPHIBIANS**

**OLEKSII MARUSHCHAK**

I. I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Bohdan Khmelnytsky St. 15, Kyiv, Ukraine

**OKSANA NEKRASOVA**

I. I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Bohdan Khmelnytsky St. 15, Kyiv, Ukraine

**VOLODYMYR TYTAR**

I. I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Bohdan Khmelnytsky St. 15, Kyiv, Ukraine

**MIHAILS PUPINS**

Daugavpils University, Institute of Life Sciences and Technologies, Department of Ecology. Parades Street 1A, Daugavpils, Latvia

**OLEKSANDRA OSKYRKO**

Taras Shevchenko National University of Kyiv, Educational and Scientific Center "Institute of Biology and Medicine", Academica Hlushkova ave. 2, Kyiv, Ukraine

The human impact on ecosystems and organisms has drawn attention to studies of amphibian anomalies as signals of environmental distress (Dubois, Ohler, 2018). Because of their complex life cycle, their direct contact with water, soil and air at all stages of their development, many factors have the potential to cause anomalies in amphibians (Henle et al., 2017). In this communication we focus on color anomalies recorded in Ukraine. Up to now in our georeferenced database there are 50 such records involving 8 amphibian species. Using a GIS approach, we made an attempt to link these anomalies with certain features of the environment which may shed more light on their origin. We used the Global Agro-Ecological Zones (GAEZ) database for characterizing climate, soil and terrain conditions, and land cover. The Human Footprint (HF) data set (Sanderson et al., 2002) was taken as a measure of anthropogenic impact. The HF layer combines layers representing population density, land use and infrastructure (built-up areas, night-time lights etc.), and human access (roads, railroads etc.). HF score ranges from 0 to 100. The background consisted of 1000 random points. Departures from randomness were found for the HF ( $38.3 \pm 1.6$  for color anomalies against  $34.1 \pm 0.3$  for randomly selected points;  $p=0.013$ ) and the GAEZ Dominant land cover pattern (45% of color anomalies recorded in anthropogenic land cover associations against a random 19%). Amongst climate, soil and terrain conditions non-randomness was discovered only for such temperature-related features: Reference length of the growing period, Reference evapotranspiration, Seasonal precipitation/potential evapotranspiration ratio (October-March).

**HISTOLOGICAL STUDY OF STOMACH AND LIVER OF *Lyciasalamandra fazilae***

EYUP BAŞKALE

Pamukkale University, Faculty of Arts and Science, Department of Biology, Denizli, Turkey

MÜGE GİDİŞ

Kütahya Dumlupınar University, Faculty of Arts and Science, Department of Biochemistry, Kutahya, Turkey

Histological examinations were conducted in order to describe the tissues on the stomach and liver of the critically endangered *Lyciasalamandra fazilae* (Başoğlu and Atatür, 1974) We examined two adult females, two adult males and one juvenile from Dalyan, Turkey and one adult male and one juvenile from Marmaris, Turkey. Tissue samples were taken from the freshly dead animals found on the road during the night field work. They were taken into alcohol for the histological studies. Tissue samples were fixed in Bouin's solution and routine histological techniques were applied. Hematoxylin and Eosin stains were used to identify cells and structures of the tissues. In the liver parenchyma, melanin containing cells were detected. In the liver parenchyma of all tissues examined, a large quantity of melanomacrophages centers. There is a lot of pigmentation in the hepatic parenchyma. Stomach is lined with columnar epithelial cells and glandular epithelium contained with gastric glands. Mucosa layers were observed as tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. There was no significant difference between males and females in mucosa layers where gastric glands are situated. The histological structure of stomach and liver of *L. fazilae* between males and females and between adults and juveniles were found to be mostly similar. Results from the literature review has indicated that both the stomach and liver histology show a similarity with the other Urodela species.

**INVESTIGATION OF THE SEXUAL DIMORPHISM BY ASSESSING  
KERATINIZED SPINES IN *Bufo bufo* SPECIES GROUP**

CANTEKİN DURSUN

Recep Tayyip Erdogan University, Rize, Turkey

NURHAYAT ÖZDEMİR

Recep Tayyip Erdogan University, Rize, Turkey

SERKAN GÜL

Recep Tayyip Erdogan University, Rize, Turkey

BİLAL KUTRUP

Karadeniz Technical University, Trabzon, Turkey

NAZAN ÜZÜM

Aydın Adnan Menderes University, Aydın, Turkey

Sexual dimorphism is a basic feature of animals which may result from sexual or natural selection. Amphibians are model organisms for testing sexual dimorphism due to diversity of their morphologies and life styles. Additionally, many secondary sexual characters may be easily observed such as dagger-like spines on the arms or chest, and enlarged oral tusks. In this study, we aimed at testing sexual dimorphism in the common toad species group (*Bufo bufo*) using keratinized spines. A total of 193 (97♂, 96♀) specimens were sampled from 25 different localities in Turkey. Photos were taken of the lateral side of the head for each individual by digital camera and they were scored for the presence of spines on the cheek with the use of five classes following previous studies. Afterwards, data were numerically coded and entered into IBM SPSS v21. To test for differences between the sexes in terms of keratinized spines, Chi-square test was used. According to the test results, a statistically significant difference was determined between sexes in terms of keratinized spines ( $\chi^2 = 78.304$ ,  $df = 4$ ,  $P < 0.05$ ). There were no keratinized spines in 65 male individuals, and 27 male individuals had lightly keratinized spines (in total %94.8), whereas almost half of the female individuals (%49) had keratinized spines on the medium level. In addition, only 12 female individuals (%12.5) were observed without keratinized spines. Consequently, it was seen that females have more keratinized spines than males which may reflect sexual dimorphism. This study was supported by TÜBİTAK under project number 114Z823.

**THERMAL PREFERENCE AND EVAPORATIVE WATER LOSS RATES IN THE  
NORTHWEST PERIPHERAL POPULATION OF THE MEADOW LIZARD  
(*Darevskia praticola*)**

JELENA ĆOROVIĆ

Institute for Biological Research „Siniša Stanković“, University of Belgrade, Belgrade, Serbia

BOGDAN JOVANOVIĆ

Institute for Biological Research „Siniša Stanković“, University of Belgrade, Belgrade, Serbia

JELKA CRNOBRNJA-ISAILOVIĆ

Institute for Biological Research „Siniša Stanković“, University of Belgrade, Belgrade, Serbia

Faculty of Sciences and Mathematics, University of Niš, Niš, Serbia

Many reptiles actively regulate their body temperature and, particularly during thermoregulation, they suffer evaporative water loss (TEWL). The intensity of water loss depends on multiple factors, including body temperature of the individual, its dehydration status, the exposure to radiative heat, air humidity and wind speed. As evaporation increases with temperature the EWL could constrain the activity of ectotherms when water is not available. With recent climate change an integrated approach to heat and water budgets is necessary to understand the present and future distribution limits of species. The thermal preference of a species can remain similar under different environmental conditions and over time, or it can change depending on a variety of factors. Also, species living under different desiccation regimes are differently adapted to resist water loss. We observed two eco-physiological traits of the meadow lizard (*Darevskia praticola*) at the western edge of the distribution area: preferred body temperature ( $T_{\text{pref}}$ ) with set-point range ( $T_{\text{set}}$ ), and EWL rates (instantaneous evaporative water loss –  $EWL_i$  and accumulated evaporative water loss –  $EWL_a$ ). Our findings showed that the thermal preference of the meadow lizard was conserved within the same sex and season, as  $T_{\text{pref}}$  and  $T_{\text{set}}$  were almost identical for males in the spring of 2014 and 2018 ( $T_{\text{pref}}$  2014: 22.1°C-35.4°C and  $T_{\text{pref}}$  2018: 22.8°C-35.3°C;  $T_{\text{set}}$  2014: 27.8°C-31.4°C and  $T_{\text{set}}$  2018: 27.7°C-31.4°C).  $EWL_i$  varied and it was highest at the beginning and lowest at the end of the experiment. The lizards lost averagely 2.8% of body weight (total EWL). This low TEWL value is peculiar, given the species' preference to humid habitats, and presumed sensibility to water loss.

**A MORPHOMETRIC STUDY OF THE COMMON CHAMELEON  
(*Chamaeleo chamaeleon*) IN SAMOS ISLAND, GREECE**

FRANCISCO JAVIER FALQUINA

Archipelagos Institute of Marine Conservation, Samos, Greece

GUIDO PIETROLUONGO

Archipelagos Institute of Marine Conservation, Samos, Greece

JASON NAGRO

Archipelagos Institute of Marine Conservation, Samos, Greece

ANASTASIA. MILIOU

Archipelagos Institute of Marine Conservation, Samos, Greece

The Common chameleon (*Chamaeleo chamaeleon*) is a reptile species found in Southern Europe, Northern Africa and Southwestern Asia. Its distribution in Greece includes the Aegean islands of Samos. The aim of this study is to improve the understanding of the morphology of the chameleon native population. Data were collected in 2016 and 2018 in Southeastern Samos. Nocturnal transects were carried out throughout five different habitats: mixed forest, coastal, riverbed, olive grove, and marsh. Specimen sex, age, snout-ventral length (SVL), tail length (TL) and total body length (TLE) were recorded. A total of 157 chameleons were observed: 79 adults, 16 juveniles, 44 newborns and 18 unknowns. No sex ratio differences were found. The largest chameleon observed was a female of 342.48 mm length. The mean TLE was 263.35 mm for males and 244.67 mm for females. The mean SVL was 129.46 for males and 119.07 mm for females whilst the mean TL was 133.89 mm for males and 125.60 mm for females. These results may suggest this population is larger in Samos compare to other locations such as Anatolia, Algeria or Spain. *C. chamaeleon* in Samos appears to have longer TL than SVL, unlike Spain where the opposite has been observed. Males SVL showed to be larger than females, unlike previous studies on the island. Long-term data collection will be necessary to ascertain whether the morphometry of *C. chamaeleon* varies according to habitat type or environmental changes induced by climate change.

**IN YOUR OWN SKIN: THE ROLE OF SKIN PEPTIDES IN AMPHIBIANS**

ILARIA DEMORI

University of Genoa-DISTAV, Genoa, Italy

ZEINAB EL RASHED

University of Genoa-DISTAV, Genoa, Italy

SARA FERRANDO

University of Genoa-DISTAV, Genoa, Italy

ELENA GRASSELLI

University of Genoa-DISTAV, Genoa, Italy

In vertebrates, a keratinized tegument appeared for the first time in amphibians, giving these animals the chance to permanently abandon the aquatic environment and become fully terrestrial. In adult amphibians skin has a multitude of functions, such as respiration, osmoregulation, defense and thermoregulation. The amphibian dermis is constellated by a plethora of mucous and poison/serous/granular glands. Mucous glands produce heavily glycosylated mucins and mucopolysaccharides, which counteract water loss by maintaining skin humidity. Poison/serous/granular glands secrete various poisonous compounds as a defense mechanism from predators. These glands are also responsible for the production of an array of proteins such as immunoglobulins, lysozymes and neuropeptides in addition to skin peptides. Peptides protect the skin from environmental and pathogenic insults and exert many other biological effects. In fact, each species produces its own specific set of peptides that fulfill many different functions from myotropical to wound healing. This latter function may be relevant in the tolerance of fungal skin infections, in particular those caused by the chytrids *Batrachochytrium dendrobatidis* and *B. salmandrivorans*.

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**PANCREATIC ISLET FORMATION IN SQUAMATES**

MAGDALENA KOWALSKA

Department of Animal Histology and Embryology, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland;  
magdalena.kowalska@us.edu.pl

PAWEŁ KACZMAREK

Department of Animal Histology and Embryology, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland

WERONIKA RUPIK

Department of Animal Histology and Embryology, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland

Pancreas is an important gland in vertebrates which contains two distinct parts: the endocrine and exocrine one. The endocrine part is formed by pancreatic islets containing endocrine cells which release hormones. The aim of this study was to investigate differentiation of the pancreatic islets in squamates using light and transmission electron microscopy with special focus on the way of islet formation, cell arrangement within islets and islet origin. In studied representants of Squamata first generation of pancreatic islets started to differentiate just after egg laying in this way that precursors of endocrine cells formed large agglomerates in the dorsal pancreatic bud. Then, agglomerates were divided into smaller units to form first islets. From developmental stage VI till hatching second generation of islets were formed from the walls of pancreatic ducts. Precursors of endocrine cells escaped from progenitor layer located in duct walls in the proces of budding and underwent incomplete delamination. Subsequently, they formed two types of small cell agglomerations on the pancreatic duct walls: comprising A and D cells or B cells only. In the next step of islet formation these cell groups joined together to form larger mature islets followed by rearrangement endocrine cells within islets. During the entire embryonic development pancreatic islets in studied species were formed mainly by A cells and at the islet periphery single B, D and PP cells were found. This arrangement of pancreatic endocrine cells is different from that described in most vertebrate species.



**ULTRASTRUCTURE OF DEVELOPING RETINA IN BROWN ANOLE (*Anolis sagrei*)**

MAGDALENA KOWALSKA

Department of Animal Histology and Embryology, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland;  
magdalena.kowalska@us.edu.pl

DOMINIKA KWIECIŃSKA

Department of Animal Histology and Embryology, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland

WERONIKA RUPIK

Department of Animal Histology and Embryology, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland

Anoline lizards are diurnally active, visually oriented animals possessing an excellent, high-acuity visual system. The retina of these lizards similarly as in other vertebrates comprises several major cell types and most important of them are the light-sensitive cells - photoreceptors. The aim of this study was to investigate development of the retina of brown anole using transmission electron microscopy with special focus of photoreceptor cell differentiation. In retina of brown anole photoreceptor cells started to differentiate just after egg laying. During retina differentiation of this species 4 types of photoreceptors were found. There were small single cone, double cone, short rod and long rod. Studying photoreceptor cells contained outer segment composed of numerous photosensitive disks and inner segment that comprised: an oil droplet, an ellipsoid, a paraboloid and the nucleus located proximal to the outer limiting membrane. All photoreceptor cells apart from the accessory member of the double cone contained an oil droplet. The large ellipsoid body was observed at the apex of the photoreceptor inner segment, immediately vitreal to the oil droplet and contained mitochondria with small quantity of mitochondrial granules. The paraboloid was found in all single cones, and double cones. In brown anole two types of paraboloid were found. One consisted of granules of glycogen and the other contained tubular membrane continuous with the rough endoplasmic reticulum. The nucleus was surrounded by a thin rim of cytoplasm and was connected to the synaptic pedicle by an axon.

**PRELIMINARY RESULTS ON THE MORPHOLOGY COMPARISON AND SEXUAL DIMORPHISM OF *Mauremys rivulata* AND *Emys orbicularis* ON THE ISLAND OF SAMOS, GREECE**

JASON NAGRO

Archipelagos Institute of Marine Conservation, Samos, Greece

GUIDO PIETROLUONGO

Archipelagos Institute of Marine Conservation, Samos, Greece

ANASTASIA MILIOU

Archipelagos Institute of Marine Conservation, Samos, Greece

FRANCISCO JAVIER FALQUINA

Archipelagos Institute of Marine Conservation, Samos, Greece

The Greek island of Samos, in the Northeastern Aegean Sea, hosts one of the most diverse herpetofauna of all Greek islands. Nevertheless, there is little literature about terrapins on the island. The aim of the study was to investigate the morphology of the *Mauremys rivulata* and *Emys orbicularis* species and to determine if sexual dimorphism was present. The study area was located in the Mesokampos marsh, Southeastern Samos. Seven morphometric measurements were collected on both *M. rivulata* (n=35) and *E. orbicularis* (n=19): curved carapace length (CCL), curved carapace width (CCW), plastron length (PL), plastron width (PW), bridge (B), head to tail length (HTT), and tail length (TL). For both species, values for these features were not significant between sexes. For interspecies comparison, sexes were combined for both species and the analysis showed that CCW and TL (two-sampled t-test, p-value=0.005 and p=0.006, respectively) were larger in *E. orbicularis* than *M. rivulata*. The mean HTT for *M. rivulata* were 155.0 mm (males) and 191.4 mm (females) whilst for *E. orbicularis* were 196.5 mm (males) and 197.6 mm (females). Further research could be carried out on the behaviour, and reproductive biology of these terrapin species, in order to apply the appropriate conservation measures.

**ECOTOURISM AFFECTS THE PHYSIOLOGICAL STRESS RESPONSE OF  
CARPETAN ROCK LIZARDS**

GONZALO RODRÍGUEZ-RUIZ

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

MARÍA MATEO-GAITÁN

Universidad Autónoma de Madrid, Madrid, Spain

ALICIA UCEDA-HERAS

Universidad Autónoma de Madrid, Madrid, Spain

ISABEL BARJA

Universidad Autónoma de Madrid, Madrid, Spain

JOSÉ MARTÍN

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

Ecotourism is a trendy activity that, even if can be responsible with the protection of the environment, produces inevitable negative consequences and impacts in nature. For example, the presence of people hiking could be considered as an increase in predation risk by many animals. The encounter between people and animals could trigger costly antipredatory and escape behaviours and increase the physiological stress response. We studied whether the levels of ecotourism in the Guadarrama National Park (Madrid, Spain) had a reflection on the stress response of fauna. We used the threatened Carpetan rock lizard (*Iberolacerta cyreni*) as a species model and compared the amount of corticosterone metabolites in the faeces as a non-invasive measure of the stress levels in lizards from two natural areas with different degree of human presence. We examined the effects of habitat characteristics and conservation state on these stress responses. We also made an experimental approach by simulating two different levels of human disturbance in captivity conditions. The results showed a higher level of corticosterone metabolites in faeces of lizards from areas or treatments with higher human presence and closer to altered habitats. Therefore, touristic pressure could constitute a stressor agent for threatened animals such as the Carpetan rock lizard.

**EFFECTS OF TEMPERATURE INCREASE ON HATCHING RATE,  
DEVELOPMENT AND SURVIVAL OF NEOTROPICAL ANURAN EMBRYOS AND  
LARVAE**

DAVID MINÁ HAFNER

Programa de Pós-Graduação em Zoologia, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

LEILDO M. CARILLO FILHO

Programa de Pós-Graduação em Zoologia, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

RENAN NUNES COSTA

Programa de Pós-Graduação em Sistemas Aquáticos Tropicais, Universidade Estadual de Santa Cruz, Ilhéus, Brazil

MIRCO SOLÉ

Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Brazil and Herpetology Section,  
Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

Amphibians, being ectothermic, are even more susceptible to environmental instabilities than any other tetrapods, and temperature has a great influence on their ecology and biology. Unlike adults, eggs and larvae are restricted to their native microhabitat and therefore are exposed to stressors associated with the aquatic environment. We analyzed whether the increase in water temperature influences the rate of hatching, survival and development of Neotropical anuran eggs. Two experiments were carried out, for which the clutches were collected by active search: experiment I - *Dendropsophus haddadi*, *Sphaenorhynchus prasinus* and *Scinax juncae* - and experiment II - *Rhinella crucifer*. The clutches were placed separately in plastic bags and transported to the laboratory for acclimation and beginning of the experiment. In experiment I in the treatment unit there was 100% of clutch mortality, therefore, only in the control unit eggs hatched and proceeded with their development. In experiment II we observed that high temperature affected the development stage and the tadpole length, resulting in individuals in more advanced stages and larger sizes for those of different stages, and smaller for comparisons among the same tadpoles of the same stages. In the control unit, the eggs hatched and continued with their normal development. High temperature did not affect hatching rates and survival of tadpoles. The thermal tolerances of tropical species are low and several are already living near the maximal values they manage to support. To guarantee the survival of their populations information on ecophysiological necessities of every development stage is dearly needed.

**PHENOTYPIC MUTATIONS OF ANURA SAMPLED IN CHERNOBYL  
EXCLUSION ZONE (CEZ)**

STELLA CORONA

Dipartimento di Scienze della Terra e dell'Ambiente, Università di Pavia, via Ferrata 9, 27100 Pavia, Italy

ADRIANA BELLATI

Dipartimento di Scienze della Terra e dell'Ambiente, Università di Pavia, via Ferrata 9, 27100 Pavia, Italy

SERGII OBRIZAN

Chornobyl radiation and ecological biosphere reserve, Chornobyl Ukraine

26<sup>th</sup> April 1986 at Chernobyl, Ukraine, there was the biggest nuclear disaster in history, from that moment all the inhabitants were evacuated. Many studies have been carried out on the effects of radiation on the human population, but little is known about reptiles and amphibians. This study was carried out on amphibians, which live in close contact with the most contaminated environments, i.e. water bodies and soil, therefore suffering a doubled exposure to radiations.

We performed captures of different life stages in the Chernobyl Exclusion Zone (CEZ) to detect aberrant phenotypic traits (malformations, behaviours, bioacoustics), genetic variation, and presence of viruses (*Ranavirus*) or fungi (*Batrachochytrium dendrobatidis*).

The study focused on the following Anura species, the European tree frog (*Hyla intermedia*), the Common toad (*Bufo bufo*), the European green toad (*Bufo viridis*), the Garlic toad (*Pelobates fuscus*), the European fire-bellied toad (*Bombina bombina*), the Marsh frog (*Pelophylax ridibundus*), the Levant water frog (*Pelophylax bedriagae*), the Edible frog (*Pelophylax kl. esculentus*), and the Pool frog (*Pelophylax lessonae*). Preliminary analyses detected the effect of radiation mainly on phenotypic and behavioural traits, but further studies will have to be carried out.

**ON THE REPRODUCTIVE BIOLOGY AND HEMIPENIAL MORPHOLOGY OF  
THE MEADOW LIZARD (*Darevskia praticola* (EVERSMANN, 1834)) (SQUAMATA:  
LACERTIDAE) IN BULGARIA**

VLADISLAV S. VERGILOV

National Museum of Natural History, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel Blvd, 1000 Sofia, Bulgaria

BOYAN ZLATKOV

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel Blvd., 1000 Sofia,  
Bulgaria

EMILIYA VACHEVA

National Museum of Natural History, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel Blvd, 1000 Sofia, Bulgaria, e-mail emilia.vacheva@gmail.com

The Meadow lizard (*Darevskia praticola* s.l.) is a small-sized forest lizard and is one of the most poorly studied species in Europe. The taxonomical status is still in debate, while molecular data suggests two main phylogenetic lineages, Balkan and Caucasian. Until now, there are no particular studies on the biology and ecology of *D. praticola* not only in Bulgaria, but also in the entire range of distribution of the species.

The studied population was located in Sredna Gora Mts, Bulgaria. The specimens were collected from April to October under permit №656/08.12.2015 from the Bulgarian Ministry of Environment and Waters. A standard histological method revealed the gonadal development in both sexes and different aspects of the onset of maturity, spermiogenesis and egg laying. The hemipenial morphology was examined for the first time in a species of this genus.

The results of the current study could be essential in thorough understanding of the biology and life cycle of this elusive lizard species and could have a taxonomical implication for clarifying the taxonomical position of this species within genus *Darevskia*.

**TESTING THE HYBRID SUPERIORITY HYPOTHESIS IN CRESTED AND  
MARBLED NEWTS**

DAN COGĂLNICEANU

Faculty of Natural and Agricultural Sciences, Ovidius University, Constanța 900470, Romania

FLORINA STĂNESCU

Faculty of Natural and Agricultural Sciences, Ovidius University, Constanța 900470, Romania

JAN ARNTZEN

Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands

We tested the hybrid superiority hypothesis in the zone of overlap and hybridization of the newts *Triturus cristatus* and *T. marmoratus*. To do so, we compared size, age and growth-related parameters in F<sub>1</sub> hybrids and both parental species in Mayenne, France. We found significant differences in snout-vent length (SVL), body mass and average lifespan between the parental species and hybrids, all three parameters showing a similar pattern, increasing from *T. cristatus* < *T. marmoratus* < hybrids. The relation between age and SVL fitted von Bertalanffy's growth model and showed that SVL<sub>max</sub> was significantly larger in hybrids than in the parental species, while the growth coefficient was lower in hybrids and *T. marmoratus* than in *T. cristatus*. *Triturus cristatus* appears to be the better competitor, since it attains sexual maturity faster and thus achieves more annual breeding opportunities. At the evolutionary level, the observed heterosis appears not to have further consequences as the hybrids are largely infertile. Our results support the hypothesis raised for the genus *Triturus*, that infertile hybrids allocate resources to growth.

**REPRODUCTIVE POTENTIALS OF TWO *Triturus* SPECIES FROM THEIR HYBRID ZONE**

TIJANA VUČIĆ

Faculty of Biology University of Belgrade, Belgrade, Serbia  
Institute for Biological Research “Siniša Stanković” University of Belgrade, Belgrade, Serbia

SONJA NIKOLIĆ

Faculty of Biology University of Belgrade, Belgrade, Serbia

JOVANA JOVANOVIĆ

Faculty of Biology University of Belgrade, Belgrade, Serbia

ANA IVANOVIĆ

Faculty of Biology University of Belgrade, Belgrade, Serbia

MILENA CVIJANOVIĆ

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

We explored some of the life history traits of *Triturus ivanbureschi* and *T. macedonicus*. These two species hybridize and form a large introgressive zone in central Balkan Peninsula which follows the specific species displacement scenario: *Triturus macedonicus* widens its range and transects the range of *T. ivanbureschi*. One of the important questions is whether, in the hybrid zone, one species has advantage over the other in reproduction or survival. During three consecutive years, we compared reproductive potentials of the two species in common garden experiments. The females of both species were introduced both to conspecific males and males of the other species. We monitored their reproductive success: proportions of egg-laying females, the total number of deposited eggs, and dynamics and duration of oviposition. Our results indicate that fecundity is largely influenced by environmental factors as well as females' affinities towards males, but specific differences in reproductive potential between the species were not found.



**OPTIMIZING THE *Bombina* TRANSCRIPTOME FOR STUDYING  
HYBRIDIZATION IN FIRE-BELLIED TOADS**

CLAUS G. ZACHO

Department of Biology, University of Copenhagen, Copenhagen, Denmark

JUDIT VÖRÖS

Department of Zoology, Hungarian Natural History Museum, Hungary

SHYAM GOPALAKRISHNAN

Department of Biology, University of Copenhagen, Copenhagen, Denmark

RASMUS NIELSEN

Department of Integrative Biology, UC-Berkeley, US

MORTEN E. ALLENTOFT

Department of Biology, University of Copenhagen, Copenhagen, Denmark

A well-characterized hybrid zone between European fire-bellied toad (*Bombina bombina*) and yellow-bellied toad (*B. variegata*) extends across parts of Central Europe and offers an excellent opportunity to gain genomic insights into the selection processes that maintain species boundary. By sequencing 150 full exomes from individuals sampled across a hybridization zone in Hungary, we will use novel admixture mapping methods to identify genes that control colour phenotypes being under divergent selection on either side of the hybridization zone. As a first crucial step we have assessed a previously published *B. bombina* transcriptome for the presence of known amphibian genes - including color genes - thus ensuring the usage of this transcriptome for capture design. First, we annotated the *Bombina* transcriptome using the *Xenopus tropicalis* annotations, resulting in 9888 transcripts of previously described amphibian genes. These were combined with 4987 unannotated transcripts that were found to have amphibian hits by a Blastn search. Our final dataset for capture design included 13.601 contigs, covering 22,947,886 bases in total. Importantly, with this annotation we could confirm that the final array included 91 genes that is known to be involved in amphibian skin colouring. However, several key colour genes, including MC1R, ASIP and pax7 was not found in the published *Bombina* transcriptome, and these were therefore added from other amphibian databases. We here present the bioinformatical pipeline we have used for this assessment, present our results, and describe how this will now be used as a foundation to study *Bombina* hybridization at a genomic level.

**NEW *Diplocynodon* REMAINS FROM THE LOWER MIOCENE  
SITE OF AHNÍKOV/MERKUR MINE  
(NORTH-WEST BOHEMIA, CZECH REPUBLIC)**

MILAN CHROUST

Czech Geological Survey, Collections Department, Klárov 3, 118 21, Prague, Czech Republic  
Charles University, Faculty of Sciences, Institute of Geology and Palaeontology, Albertov 6, 128 43, Prague, Czech Republic

MARTIN IVANOV

Masaryk University, Faculty of Sciences, Department of Geological Sciences, Kotlářská 267/2, 611 37 Brno, Czech Republic

ANDREJ ČERNÁNSKÝ

Comenius University in Bratislava, Faculty of Natural Sciences, Department of Ecology, Mlynská dolina, Ilkovičova 6, 842  
15 Bratislava, Slovak Republic

JOSEP FORTUNY

Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Edifici ICTA-ICP, c/ Columnes s/n,  
Campus de la UAB, 08193 Cerdanyola del Vallès, Spain

MARTIN MAZUCH

Charles University, Faculty of Sciences, Institute of Geology and Palaeontology, Albertov 6, 128 43, Prague, Czech Republic

ÁNGEL H. LUJÁN

Masaryk University, Faculty of Sciences, Department of Geological Sciences, Kotlářská 267/2, 611 37 Brno, Czech Republic

Comenius University in Bratislava, Faculty of Natural Sciences, Department of Ecology, Mlynská dolina, Ilkovičova 6, 842  
15 Bratislava, Slovak Republic

Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Edifici ICTA-ICP, c/ Columnes s/n,  
Campus de la UAB, 08193 Cerdanyola del Vallès, Spain

First alligatoroid *Diplocynodon* remains from the Ahníkov/Merkur Mine site (A/MM) are described here. A/MM is the richest fossil site of the Most Basin (Eger Graben, Czech Republic), including more than 150 taxa of fossil vertebrates reported to date. Specifically, more than 300 remains of fossil crocodylians have been collected, but they have never been studied in detail. These crocodylian remains are the largest sample for the Czechia. A/MM is dated to the early Miocene MN3a (early Burdigalian) of the European Neogene Mammal biochronological system. The material belongs to several individuals consisting of isolated teeth, fragments of both cranial and postcranial bones. This material is herein referred to *Diplocynodon cf. ratelii* according to the length of the dentary symphysis (reaching to the third or fourth dentary alveoli) as well as the position of the splenial (excluded from the symphysis and the anterior ventral tip of the splenial is anteriorly longer than the dorsal one). Due to a small size and unfinished closure of bone sutures, all recovered specimens of *Diplocynodon* appear to represent a juvenile stage. We infer to the *Diplocynodon* genus a similar ecological behavior like extant alligators, where the juveniles prefer wetlands, instead of open waters. This fact is congruent with the proposed paleoenvironment reconstruction where the site has been interpreted as a shore of a lake with a terrestrial influence. Fossil remains of *Diplocynodon* from A/MM are the most northeast occurrence of this taxon in Europe, and therefore, extend the paleogeographic distribution during early Miocene in Central Europe.

**ANATOMICAL NETWORK ANALYSIS OF THE GEKKOTAN SKULL**

ALEXANDRA HERRERA  
Sam Houston State University, Huntsville, USA  
BORJA ESTEVE-ALTAVA  
Pompeu Fabra University, Barcelona, Spain  
JUAN D. DAZA  
Sam Houston State University, Huntsville, USA  
AARON M. BAUER  
Villanova University, Villanova, USA

Anatomical network analysis (AnNA) is a powerful method that allows the quantification of modularity, integration, complexity, and evolvability. In this study, we applied this method to the gekkotan skull, using the suturing patterns across virtually all genera. Preliminary observations indicate that the gekkotan skull exhibits a remarkable variation among sutures. The anatomical networks of species vary due to fusion and loss of bone elements. The maxilla contacts the largest number of bones in the skull. There are many groups in which the bones from the dorsal mid-line of the skull are paired or not (premaxillae, nasals, frontals and parietals). Some bones that were not present in all species include the lachrymal, supratemporal, the quadrate sesamoid, and a neomorphic element exclusive to *Nephrurus asper*. The jaw showed variation in the number of bone elements among gekkotans, especially in the composition of the compound bone (variable in its inclusion of the angular and surangular) and the presence or absence of the splenial bone. While overall the Gekkota shows a generalized pattern, the topographic relationships among the bones varies considerably, offering a good system to explore structural variation at a macroevolutionary scale.

**THE SKELETON OF THE BURROWING AFRICAN ANURAN (*Hemissus marmoratus*, NEOBATRACHIA: HEMISOTIDAE)**

MATTEO PILI

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

ANDREA VILLA

Bayerische Staatssammlung für Paläontologie und Geologie, München, Germany

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

THALASSA MATTHEWS

Centre of Excellence for Palaeontology, Iziko Museums of South Africa, Cape Town, South Africa

MASSIMO DELFINO

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Edifici Z (ICTA-ICP), Barcelona, Spain

Burrowing anurans are characterized by several osteological peculiarities related to their ecology. The skeletons of five specimens of the African anuran *Hemissus marmoratus* (Hemisotidae), two males (KNM-OR 479; ZR-13981) and three females (MDHC 411, 445; ZR-43835) were studied. Thanks to this material (some of it represented by a dry, disarticulated skeleton and some of it CT scanned), it has been possible to describe in detail the morphology of each skeletal element, highlighting the peculiar characteristics of this species and comparing them to other bizarre anurans with peculiar adaptations to digging. Hence we have deduced the implications of the skeletal anatomy on the ecology of the species and on its behavior, particularly in relation to the locomotion and excavation activity. The most derived skeletal regions are: the skull, characterized by a marked process of fusion of originally separated bones (nasals and sphenethmoid fused in the nasoethmoid, and frontoparietal, prootic-exoccipitals and parasphenoid fused in a posterior skull complex); the pectoral girdle, characterized by the fusion of scapula, clavicle and coracoid. The humeri of the male specimens are characterized by a deep and well-defined longitudinal groove proximally developed on the medial surface (medial to the ventral crest) and distally extending along the ventral surface of the shaft. The groove is not so evident in the female specimens, suggesting that this could be a sexually dimorphic character.

**SKULL DEVELOPMENT IN COMMON TOADS: A 3D GEOMETRIC  
MORPHOMETRIC ANALYSIS**

GIOVANNI SANNA

University of Bologna, Bologna, Italy

Postmetamorphic cranial ontogeny was analyzed in the closely related common toad (*Bufo bufo*) and Western common toad (*Bufo spinosus*), using micro-computed tomography and applying geometric morphometrics on 3D landmark data. These powerful tools allow for a detailed quantification of shape variation. Allometry appears to play a major role in the postmetamorphic development of both species. While growing, the skull broadens considerably, becoming proportionally shorter and lower. The allometric trajectories do exhibit some interspecific differences, which are reflected in the morphological disparity that progressively sharpens over growth. In fact, mature *B. bufo* can be distinguished by a longer skull and a lower snout than mature *B. spinosus*. Yet, such differences remain more subtle than the shared ontogenetic constraints.

**SKULL OSTEOLOGY OF *Vipera walser*: DESCRIPTION, VARIABILITY, ONTOGENY AND DIAGNOSTIC CHARACTERS IN THE FRAME OF THE ITALIAN VIPERS**

SIMONE MATTEO SEGHETTI

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

ANDREA VILLA

Bayerische Staatssammlung für Paläontologie und Geologie, München, Germany

Dipartimento di Scienze della Terra, Università di Torino, Italy

EMANUEL TSCHOPP

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

Division of Paleontology, American Museum of Natural History, New York City, NY, USA

Universidade Nova de Lisboa, Faculdade de Ciências e Tecnologia, Caparica, Portugal

Museu da Lourinhã, Lourinhã, Portugal

FEDERICO BERNARDINI

Centro Fermi, Museo Storico della Fisica e Centro di Studi e Ricerche "Enrico Fermi", Roma, Italy.

Multidisciplinary Laboratory, the "Abdus Salam" International Centre for Theoretical Physics, Trieste, Italy

LORENZO LADDAGA

Società di Scienze Naturali del Verbano Cusio Ossola, Museo di Scienze Naturali Collegio Mellerio Rosmini, Domodossola, Italy

MAURO FANELLI

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

RENZO LEVI

Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Torino, Italy

MICHELE MENEGON

Division of Biology & Conservation Ecology, Manchester Metropolitan University, UK

PAMS Foundation, Arusha, Tanzania

MASSIMO DELFINO

Dipartimento di Scienze della Terra, Università di Torino, Torino, Italy

Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Barcelona, Spain.

*Vipera walser* is the latest described European viper. This species is endemic of a small area in the Italian Alps in Piedmont, but its closest relatives are to be sought among the Caucasian viper species. In order to provide a starting point for a phylogenetic and biogeographic investigation based on osteology that could potentially include also fossil remains, we analyzed four specimens of *V. walser* (two dry preserved, disarticulated skulls and two CT scans) and compared them with specimens of the four other Italian viper species. On the basis of these specimens we improved the diagnosis of *V. walser* and started to evaluate intraspecific variability and ontogenetic variation. All the skull bones have been described and figured in different views. The skull of *V. walser* shows a high degree of intraspecific variation, part of which is probably related to ontogeny (development of the parietal crest, development of the basioccipital process, shape of the posterior margin of the basiparasphenoid, shape of the quadrate) but it differs from that of all other Italian vipers because of the shape of the occipital crest of the supraoccipital.

**PREVALENCE AND DISTRIBUTION OF THE AMPHIBIAN PATHOGEN  
*Batrachochytrium dendrobatidis* IN ITALY**

LORENZO DONDERO  
DISTAV, University of Genova, Italy  
GIORGIA BIANCHI  
DISTAV, University of Genova, Italy  
BRYAN MORALES SANCHEZ NELSON  
DISTAV, University of Genova, Italy  
MARCO MAGGESI  
DISTAV, University of Genova, Italy  
VITTORIA MARCHIANÒ  
Parco Nazionale del Pollino  
MARCO CARAFA  
Parco Nazionale della Majella  
Servizio Biodiversità e Reti Ecologiche  
Parco Nazionale del Circeo  
ANTONIO ROMANO  
CNR-ISFOM, Ercolano Italy; MUSE Museo delle Scienze, Trento Italy  
MATTEO PERRONE  
Parco Nazionale delle Cinque Terre  
ANDREA COSTA  
DISTAV, University of Genova, Italy  
SEBASTIANO SALVIDIO  
DISTAV, University of Genova, Italy  
ELENA GRASSELLI  
DISTAV, University of Genova, Italy

The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) is a pathogen that infects keratinized parts of amphibians, such as the keratodonts in larvae and the skin in adults. Chytridiomycosis caused by *Bd* has caused mass mortality events in Australia, Central America and Spain. However, little is known about the distribution and population prevalence of *Bd* in Italy, apart from the description of a mass infection event in Sardinia and a first national synthesis. In this study we report the results of a *Bd* screening in peninsular Italy. Overall, more than 1200 individual swabs were analysed for *Bd*, by means of a real-time PCR based on SYBR Green chemistry, a molecular technique that quantifies the individual *Bd* load in genome equivalents (GEs). Overall, 14 amphibian species were screened (7 anurans and 7 urodelans), and *Bd* infected amphibians were found all along the Apennines, both in anthropic and natural habitats. At the species level *Bd* infection prevalence ranged from 0% to 25%, while at the individual level the infection load of *Bd* was generally low, ranging from 1 to 150 GEs.

**EUROPEAN COLLABORATION FOR EX\_SITU CONSERVATION OF THE STRIPED FIRE SALAMANDER, *Salamandra salamandra TERRESTRIS***

KATHLEEN PREISLER

Universität Leipzig

Institut für Biologie

Molekulare Evolution und Systematik der Tiere

Talstraße 33

04103 Leipzig

SERGÉ BOGAERTS

Lupinelaan 25

Waalre

The Netherlands

ANNEMARIEKE SPITZEN

Stichting RAVON

PO Box 1413

NL 6501 BK Nijmegen

The Netherlands

on behalf of the Ex-situ Salamandra Group

Following the introduction of the invasive chytrid fungus *Batrachochytrium salamandrivorans* (Bsal) into Europe, multiple populations of the (Striped) fire salamander (*Salamandra salamandra terrestris*) have collapsed in north-western Europe. Currently, the species is threatened with extinction in the Netherlands as various populations in Belgium and Germany. Upon the discovery of Bsal, and associated mass mortalities, a captive assurance colony was established in the Netherlands. In the face of continuous spreading of Bsal into new areas within Belgium and Germany, both countries aim to develop similar ex-situ programs.

To ensure collaboration, shared goals and to effectively share knowledge and resources, the multidisciplinary 'Ex-situ Salamandra Group' (ESG) was initiated by scientists, NGOs and zoos from the three Bsal affected countries. Close collaboration and mutual commitment between all partners involved is the strength of this group.

For this ex-situ program, it is necessary to collect available scientific knowledge on genetics, ecology and behaviour, and translate them into practical ways to keep and possibly in a later stage also breed the species. The development of a scientifically based and EAZA (European Association of Zoo's and Aquaria) approved husbandry protocol for *Salamandra s. terrestris* is a first product; in a second phase it can be expanded to other threatened European urodeles.



# Workshop: Developing a response plan to *Batrachochytrium salamandrivorans*

## THE EUROPEAN RESPONSE TO THE *Batrachochytrium salamandrivorans* INVASION IN EUROPE

LIEZE ROUFFAER

Wildlife Health Ghent, Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

FRANK PASMANS

Wildlife Health Ghent, Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

ANNEMARIEKE SPITZEN-VAN DER SLUIJS

Ravon, Nijmegen, The Netherlands

MAARTEN GILBERT

Ravon, Nijmegen, The Netherlands

RONALD ZOLLINGER

Ravon, Nijmegen, The Netherlands

ARNAUD LAUDELOUT

Natagora, Namur, Belgium

ANDREW A. CUNNINGHAM

Institute of Zoology, Zoological Society of London, London, United Kingdom

STEFAN LÖTTTERS

Biogeography Department, Faculty of Geography/Geosciences, Trier University, Trier, Germany

MICHAEL VEITH

Biogeography Department, Faculty of Geography/Geosciences, Trier University, Trier, Germany

JAIME BOSCH

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

SEBASTIANO SALVIDIO

Distav, Genoa, Italy

ELENA GRASSELLI

Distav, Genoa, Italy

CLAUDE MIAUD

Centre d'Écologie Fonctionnelle et Évolutive (CEFE), University of Montpellier, Montpellier, France

AN MARTEL

Wildlife Health Ghent, Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

THIERRY KINET

Natagora, Namur, Belgium

Forty percent of all amphibian species are threatened with extinction, with the introduction of alien invasive pathogens significantly contributing to this threat. The introduced chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) has caused the collapse of several wild salamander populations in the Netherlands, Belgium and Germany and severely threatens urodelan diversity in Europe. Mitigating the effects of *Bsal* requires a coherent, pro-active, multidisciplinary and shared approach. Therefore, a European collaboration between veterinarians, ecologists and conservationists was set in place to protect urodelan diversity in Europe (Tender issued by the European Commission (EC)).

Through this EU-Tender Project, the delineation of the current range of *Bsal* in Europe has been mapped and is continuously being updated. Measures have been and are being developed which aim at preventing further entry and spread of this amphibian pathogen into and through Europe. An early warning system that allows rapid detection of novel *Bsal* outbreaks is (being) established through the set-up of regional hotlines and a network of diagnostic laboratories (spread over Europe). Emergency action plans, both species specific and general plans, a set of short-term mitigation measures, and proof of concepts for sustainable long term mitigation measures are being developed.

An overview of the current results will be presented, including the current range of *Bsal*, the inter- and intra-European trade ban of Asian salamanders, results of the implementation of the early warning system and development of emergency action plans.

**THE FORGOTTEN HOTSPOT: A DECADE OF RESEARCH INTO THE  
HERPETOFAUNA OF THE COMOROS ARCHIPELAGO**

OLIVER HAWLITSCHKEK

Zoologische Staatssammlung (SNSB-ZSM), Münchhausenstr. 21, 81247 Munich, Germany

MARK D. SCHERZ

Zoologische Staatssammlung München (ZSM-SNSB), Münchhausenstr. 21, 81247 München, Germany

The Comoros is an archipelago of four major oceanic islands in the Western Indian Ocean. Unlike most other oceanic archipelagoes, the fauna of the Comoros remains poorly studied despite its high diversity and endemism. Today, reptiles may be the best-studied animal group of the Comoros. Reviewing my work of the last decade, I highlight the diverse biogeographic connections of the archipelago and the various colonization mechanisms of the past. Most reptile clades colonized the Comoros from the Northern tip of Madagascar, where the fast-flowing North East Madagascar Current acts as an overseas dispersal 'conveyor'. The ancestors of other reptile species colonized the Comoros from central western Madagascar, continental Africa, or the Eastern Indian Ocean. In addition to common intra-archipelago dispersal and speciation, I present possible evidence of several extinction events followed by, or following, (re-)colonization by closely related lineages from Madagascar. In the *Ebenavia inunguis* species complex of geckos, the clade inhabiting the three western islands of the Comoros is sister to all other extant lineages. Its estimated age is considerably older than the extant islands of the archipelago (13–20 vs. ca. 11 million years). Furthermore, the two species of frogs from the Comoros are among the comparatively few unambiguous examples of amphibian species endemic to oceanic islands.







## List of participants

Name	Surname	Email	Institution
Diana	Abondano Almeida	dianabondano1@gmail.com	Goethe University, Frankfurt, Germany
Maja	Ajduković	maja.ajdukovic@ibiss.bg.ac.rs	Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia
Elisa	Alari	Elisa.alari@hotmail.com	Università degli Studi di Milano, Italy
Davide	Alberti	davide.alberti@parcoforestecasentinesi.it	Parco Nazionale Foreste Casentinesi, Monte Falterona and Campigna, Italy
Morten	Allentoft	meallentoft@bio.ku.dk	University of Copenhagen, Denmark
Marko	Anđelković	marko.andjelkovic@ibiss.bg.ac.rs	Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia
Franco	Andreone	franco.andreone@regione.piemonte.it	Museo Regionale di Scienze Naturali, Torino, Italy
Bernardo	Antunes	bernardofloresantunes@gmail.com	Institute of Environmental Sciences. Jagiellonian University, Krakow, Poland
Viviana	Arguedas	viviana.arguedasporras@ucr.ac.cr	University of Costa Rica, Costa Rica
Ignazio	Avella	ignazio.avella.1990@gmail.com	Faculty of Sciences of the University of Porto, Portugal
Vasco	Avramo	vasco.avramo@uniroma1.it	University of Rome "La Sapienza", Italy
Wiesław	Babik	wieslaw.babik@uj.edu.pl	Jagiellonian University, Krakow, Poland
Bakhouche	Badis	badis_bakhouche@yahoo.fr	Dynamic Laboratory and Biodiversity, Houari Boumediene University, Belgium
Simon	Baeckens	simon.baeckens@uantwerp.be	University of Antwerp, Belgium
Luca	Baglioni	baglio96luca@gmail.com	Università degli Studi di Milano, Italy
Monika	Balogová	monika.balogova01@gmail.com	Institute of Biology and Ecology, Faculty of Science, P.J. Šafárik University, Košice, Slovakia
Federico	Banfi	federico.banfi.93@gmail.com	Biology Department, Vrije Universiteit Brussel, Brussels, Belgium
Andrea	Barbi	andrea.barbi@edu.unito.it	Università degli Studi di Torino, Italy
Marco	Barquero	marco.barquero_a@ucr.ac.cr	University of Costa Rica, Costa Rica
Benedetta	Barzaghi	benedetta.barzaghi@studenti.unimi.it	University of Milan, Italy
Eyup	Başkale	eyupbaskale@gmail.com	Pamukkale University, Turkey
Senka	Baškiera	sbaskiera@gmail.com	Masaryk University, Faculty of Science, Brno, Czech Republic
Aaron	Bauer	aaron.bauer@villanova.edu	Villanova University, Pennsylvania, United States
Adriana	Bellati	adriana.bellati@unipv.it	University of Pavia, Italy
Francesco	Belluardo	france89belluardo@gmail.com	CIBIO Centro de Investigação em Biodiversidade e Recursos Genéticos; Universidade do Porto, Portugal
Lejeune	Benjamin	benjamin.lejeune@uliege.be	University of Liège, Belgium
Emanuele	Berrilli	emanuele.berrilli@graduate.univaq.it	Università degli Studi dell'Aquila, Italy
Jean-Louis	Berthoud	jeanlouis.Berthoud@posteo.net	Universität Hohenheim / Staatliches Museum für Naturkunde Stuttgart, Germany
Wouter	Beukema	wouter.beukema@ugent.be	Ghent University, Belgium
Marta	Biaggini	marta.biaggini@virgilio.it	Museo di Storia Naturale dell'Università degli Studi di Firenze, Italy
Lilijana	Bizjak Mall	lila.bizjak@bf.uni-lj.si	University of Ljubljana, Slovenia
Vukašin	Bjelica	b1027_2018@stud.bio.bg.ac.rs	University of Belgrade, Faculty of Biology, Belgrade, Serbia
Gabriel	Blouin-Demers	gblouin@uottawa.ca	University of Ottawa, Canada

Serge	Bogaerts	s-bogaerts@hetnet.nl	Salamandervereniging/AG Urodela, the Netherlands
Lucio	Bonato	lucio.bonato@unipd.it	Università di Padova, Italy
Martin	Bonhomme	martin.bonhomme59@yahoo.fr	Parc Naturel Régional des Landes de Gascogne, Belin-Beliet, France
Danilo	Borgatti		Università degli Studi di Milano, Italy
Angela Lisa	Borghi	angelalisa.borghi01@universitadipavia.it	University of Pavia, Italy
Wolfgang	Böhme	W.Boehme@leibniz-zfmk.de	Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Adenauerallee 160. D-53113 Bonn, Germany
Katarina	Breka	katarina.breka@bio.bg.ac.rs	Faculty of Biology, University of Belgrade, Serbia
Giacomo	Bruni	giacomo.b90@gmail.com	Vrije Universiteit Brussels, Belgium
Stephen	Busack	sbusack348@aol.com	North Carolina Museum of Natural History, Raleigh, NC, USA
Natalija	Čađenović	natalicadjenovic@gmail.com	The Natural History Museum of Montenegro, Montenegro
Kamil	Candan	kamil.candan@deu.edu.tr	Dokuz Eylül University, İzmir/Balçova, Turkey
Rafaela	Cândido De França	rafaela.franca.ufpb@gmail.com	Universidade Estadual de Santa Cruz, Brasil
Stefano	Canessa	canessa.stefano@ugent.be	Ghent University, Belgium
Salvador	Carranza	salvicarranza@gmail.com	Institute of Evolutionary Biology (CSIC-UPF), Barcelona, Spain
Miguel Angel	Carretero	carretero@cibio.up.pt	Universidade do Porto, Portugal
Sarah	Chaloupka	sarah.chaloupka@gmail.com	University of Vienna, Austria
Darío	Chamorro	dariochamorro@uma.es	Depto. de Biología Animal, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain
Michele	Chiacchio	michele.chiacchio@ufz.de	Helmholtz, Centre for environmental research, Germany
Milan	Chroust	chrousm@natur.cuni.cz	Charles University, Prague, Czech Republic
Vincent	Clement	vincent.cle@laposte.net	Association BUFO, Strasbourg, France
Dan	Cogălniceanu	dan_cogalniceanu@yahoo.com	Faculty of Natural Sciences and Agricultural Sciences, Ovidius University, Constanța, Romania
Alan	Coladonato	alanjioele.coladonato01@universitadipavia.it	Università degli Studi di Pavia, Italy
Luca	Coppari	luca.coppari@yahoo.it	S.H.I. Sezione interregionale Umbria – Marche, Italy
Stella	Corona	stella.corona01@universitadipavia.it	Università degli studi di Pavia, Italy
Jelena	Ćorović	jelena.corovic@ibiss.bg.ac.rs	Institute for Biological Research „Siniša Stanković“, University of Belgrade, Serbia
Claudia	Corti	claudia.corti@unifi.it	Museo di Storia Naturale dell'Università di Firenze, Italy
Andrea	Costa	andrea-costa-@hotmail.it	University of Genova, Italy
Jelka	Crnobrnja-Isailović	jelka.c.i@gmail.com	Institute for Biological Research “Siniša Stanković” University of Belgrade, Serbia
Angelica	Crottini	tiliquait@yahoo.it	CIBIO/InBio, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal
Pierangelo	Crucitti	info@srsn.it	Società Romana di Scienze Naturali, Roma, Italy
Milena	Cvijanović	milena.cvijanovic@ibiss.bg.ac.rs	Institute for Biological Research “Siniša Stanković” University of Belgrade, Belgrade, Serbia
Urban	Dajčman	urban.dajcman@gmail.com	University of Ljubljana, BIOTECHNICAL FACULTY, Slovenia

Andrea	Dalpasso	Andrea.dalpasso@studenti.unimi.it	Università degli Studi di Milano, Italy
Gorana	Danon	gorana.danon@gmail.com	Faculty of Biology, University of Belgrade, Serbia
Juan D.	Daza	juand.daza@gmail.com	Sam Houston State University, Huntsville, USA
Aris	Deimezis-Tsikoutas	adeime@biol.uoa.gr	Department of Biology, National and Kapodistrian University of Athens, Athens, Greece
Massimo	Delfino	massimo.delfino@unito.it	University of Torino, Italy
Daniele	Delle Monache	pentathlon91@gmail.com	Università di Bologna, Italy
Katharina	Dellefont	katharinadellefont@live.at	University of Vienna, Austria
Viola	Di Canio	Viola.dicanio@studenti.unimi.it	Università degli Studi di Milano, Italy
Anna Rita	Di Cerbo	annarita.dicerbo@gmail.com	Centro Studi Fauna Vertebrata "Luigi Cagnolaro", Società Italiana di Scienze Naturali c/o Museo Civico di Storia Naturale di Milano, Italy
Maria	Dimaki	mdim@gnhm.gr	Goulandris Natural History Museum, Athens, Greece
Inês	Dinis De Freitas	ifinesfreitas@hotmail.com	CIBIO/InBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão; Universidade do Porto, Portugal
Carolin	Dittrich	carolin.dittrich@mfh.berlin	Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany
Colin	Donihue	colindonihue@gmail.com	Washington University in St. Louis, USA
Siarhei	Drabiankou	bel_gerpology@rambler.ru	State Scientific and Production Amalgamation «Scientific and Practical Center for Bioresources» of the National Academy of Sciences of Belarus, Belarus
Charlotte	Ducotterd	charlotte.ducotterd@gmail.com	University of Lausanne, Switzerland
Katarzyna	Dudek	katarzyna2.dudek@uj.edu.pl	Jagiellonian University, Krakow, Poland
Christophe	Dufresnes	christophe.dufresnes@hotmail.fr	Department of Animal & Plant Sciences, University of Sheffield, United Kingdom; Hintermann & Weber, Montreux, Switzerland
Remi	Duguet	rduguet@expertise-ecologique.fr	Alcedo Fauna and Flora, Sanilhac, France
Cantekin	Dursun	cantekin.dursun@erdogan.edu.tr	Recep Tayyip Erdogan University, Rize, Turkey
Stefan	Einarson	einarson@brill.com	Brill, Leiden, The Netherlands
Urtzi	Enriquez-Urzelai	urtzi.enriquez@gmail.com	CIBIO/InBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal
Karin	Ernst	karin.ernst@hotmail.com	Natural History Museum Vienna, Austria
Mattia	Falaschi	matt_fala@hotmail.it	Università degli Studi di Milano, Italy
Francisco Javier	Falquina	jfalquina@hotmail.com	Archipelagos Institute of Marine Conservation, Samos, Greece
Anna	Fedorova	minihobbit29@gmail.com	Department of Zoology and Animal Ecology, V. N. Karazin Kharkiv National University, Kharkiv, Ukraine
Nathalie	Feiner	nathalie.feiner@biol.lu.se	Lund University, Lund, Sweden
Daniel	Fernández-Guiberteau	dfguiberteau@grenp.org	Grup de Recerca de l'Escola de la Natura de Paret del Vallès (GRENP), Paret del Vallès, Spain
Miguel	Ferreira	miguel.rod.ferreira@gmail.com	Faculty of Sciences of the University of Porto, Portugal
Gentile Francesco	Ficetola	francesco.ficetola@gmail.com	Università degli Studi di Milano, Italy

Jim	Foster	jim.foster@arc-trust.org	Amphibian and Reptile Conservation Trust, Bournemouth, UK
Susana	Freitas	freitas.sn@gmail.com	University of Lausanne, Switzerland
Benedetta	Gamboli	benedetta.gamboli@gmail.com	Università Roma Tre, Roma, Italy
Simone	Giachello	simone.giachello@studenti.unimi.it	Università degli Studi di Milano, Italy
Müge	Gidis	mugegidis@gmail.com	Kütahya Dumlupınar University, Faculty of Arts and Science, Department of Biochemistry, Kutahya, Turkey
Maarten	Gilbert	m.gilbert@ravon.nl	Reptile, Amphibian and Fish Conservation the Netherlands (RAVON), the Netherlands
Philipp	Ginal	philipp.ginal@gmx.de	Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany
Andrea	Gini	andreaeugenio.gini@gmail.com	Scuola Normale Superiore, Pisa, Italy
Marko	Glogoški	marko.glogoski@biol.pmf.hr	Faculty of Science, University of Zagreb, Croatia
Philippe	Golay	gogo@geneva-link.ch	Fondation Culturelle Elapsoïdea, Geneva, Switzerland
Günter	Gollmann	guenter.gollmann@univie.ac.at	University of Vienna, Austria
Ana	Golubović	golubovic.ana@bio.bg.ac.rs	Faculty of Biology, University of Belgrade, Serbia
Verónica	Gomes	veronica.a.s.g@gmail.com	CIBIO/InBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal
Duarte	Gonçalves	duartenvg@gmail.com	CIBIO/InBIO – U. Porto; CIIMAR-U.Porto, Porto, Portugal; IBE (CSIC-UPF), Barcelona, Spain
Vladislav	Gorin	gorinvlad@gmail.com	Biological Faculty of Lomonosov Moscow State University, Russia
Elisabetta	Gozzo	elisabettagozzo95@gmail.com	Università degli Studi di Milano, Italy
Elena	Grasselli	elena.grasselli@unige.it	Università degli Studi di Genova, Italy
Deso	Grégory	ahpam.contact@gmail.com	AHPAM, Orange, France
Fabio Maria	Guarino	fabio.guarino@unina.it	University of Naples Federico II, Napoli, Italy
Olivier	Guillaume	olivier.guillaume@sete.cnrs.fr	National Center for Scientific Research, Moulis, France
Daniel	Guinart Sureda	guinartsd@diba.cat	Diputacio de Barcelona , Catalonia, Spain
Vaclav	Gvozdik	vaclav.gvozdik@ivb.cz	Institute of Vertebrate Biology of the Czech Academy of Sciences, Brno, Czech Republic
Jeanette	Hall	gentiananivalis@hotmail.com	Highland Biological Recording Group, Inverness, UK
Jakob	Hallermann	hallermann@uni-hamburg.de	Cenak -Center of Natural History, Inverness, UK
Bálint	Halpern	balint.halpern@gmail.com	MME BirdLife Hungary, Budapest, Hungary
Alena Marcella	Hantzschmann	alena@uni-koblenz.de	University of Koblenz-Landau, Germany
David	Herczeg	herczegdavid88@gmail.com	Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary
Jelger	Herder	j.herder@ravon.nl	Reptile Amphibian Fish Conservation Netherlands (RAVON), the Netherlands
Anthony	Herrel	Anthony.herrel@mnhn.fr	CNRS/MNHN, Paris, France
Alexandra	Herrera	hm.alexandra@gmail.com	Sam Houston State University, Huntsville, USA
Martin	Hlubeň	hluben.martin@gmail.com	Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic
Louise	Hobin	lhobin@amphibians.org	IUCN Amphibian Red List Authority, Truro, UK
Tomáš	Holer	holer.tomas@gmail.com	Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic
Souad	Hraoui-Bloquet	sdbloquet@yahoo.com	Faculty of Sciences, Fanar campus, Lebanese University, Lebanon

Ana	Ivanovic	ana@bio.bg.ac.rs	University of Belgrade, Faculty of Biology, Belgrade, Serbia
Dušan	Jelić	Jelic.dusan@gmail.com	Croatian Institute for Biodiversity, Zagreb, Croatia
Mitali	Joshi	s6mijosh@uni-bonn.de	Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany
Olga	Jovanović Glavaš	jovanovic.olga@gmail.com	University of Osijek, Osijek, Croatia
Glos	Julian	julian.glos@uni-hamburg.de	Institute for Zoology, University of Hamburg, Germany
Jan M.	Kaczmarek	kaczmarq@gmail.com	Institute of Zoology, Poznań University of Life Sciences, Poznań, Poland
Hinrich	Kaiser	chalcopis@yahoo.com	Victor Valley College, Victorville, CA, USA
Antigoni	Kaliontzopoulou	antigoni@cibio.up.pt	CIBIO/InBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal
Ana	Kijanović	ana.kijanovic@ibiss.bg.ac.rs	Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia
Janusz	Kloskowski	janusz6kl@gmail.com	Institute of Zoology, Poznań University of Life Sciences, Poznań, Poland
Krzysztof	Kolenda	krzysztof.kolenda@uwr.edu.pl	Institute of Environmental Biology, University of Wrocław, Wrocław, Poland
Thore	Koppetsch	t.koppetsch@leibniz-zfmk.de	Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany
Yurii	Kornilev	yukornilev@gmail.com	National Museum of Natural History, Sofia, Bulgaria; Department of Integrative Zoology, Faculty of Life Sciences, University of Vienna, Vienna, Austria
Yahor	Korzun	natrix109@gmail.com	State Scientific and Production Amalgamation «Scientific and Practical Center for Bioresources» of the National Academy of Sciences of Belarus
Jan	Kovařík	jan.kovarik@naturaservis.net	NaturaServis s.r.o., Czech Republic
Magdalena	Kowalska	magdalena.kowalska@us.edu.pl	University of Silesia in Katowice, Katowice, Poland
Anna	Kozáková	anna.kozakova@naturaservis.net	NaturaServis s.r.o., Czech Republic
Antonín	Krásá	antonin.krasa@nature.cz	Praha 11-Chodov, Czech Republic
Alena	Kulikova	Elen.Kulikova@gmail.com	State Scientific and Production Amalgamation «Scientific and Practical Center for Bioresources» of the National Academy of Sciences of Belarus
Larissa	Kupriyanova	larissakup@zin.ru	Zoological Institute, Russian Academy of Sciences, Saint Petersburg, Russia
David	Lastra González	lastra_gonzalez@fzp.czu.cz	Czech University of Life Sciences in Prague, Faculty of Environmental Sciences, Prague, Czech Republic
Jörn	Laudor	joernlaudor@googlemail.com	Trier University, Germany
Boualit	Laurent	laurent.boualit@unil.ch	Université de Lausanne, Environmental Sciences, Switzerland
Christoph	Leeb	leeb@uni-landau.de	University Koblenz-Landau, Germany
Astrid	Lenisa	astrid.lenisa@studenti.unimi.it	Università degli Studi di Milano, Italy
Fulvio	Licata	fulvio.licata@gmail.com	CIBIO/InBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal
Anastasios	Limnios	tasoslimnios@gmail.com	Department of Zoology - Marine Biology Faculty of Biology National and Kapodistrian University of Athens, Athens, Greece
Konrad	Lipkowski	lipkowski@bio.uni-frankfurt.de	Johann-Wolfgang-Goethe-University, Frankfurt/M, Germany
Bianca	Lombardi	bianca.lombardi@studenti.unimi.it	Università degli Studi di Milano, Italy



Elia	Lo Parrino	elia.loparrino@studenti.unimi.it	Università degli Studi di Milano, Italy
Julia	Lopez Delgado	julialopezdelgado94@gmail.com	Leiden University, the Netherlands
Stefan	Lötters	loetters@uni-trier.de	Trier University, Germany
Nahla	Lucchini	lucchini.nahla@gmail.com	Alma Mater Studiorum - Università di Bologna, Italy
Jan-Dieter	Ludwigs	jan-dieter.ludwigs@rifcon.de	Rifcon GmbH, Hirschberg an der Bergstraße, Germany
Jennifer	Luedtke	jluedtke@amphibians.otg	IUCN Amphibian Red List Authority, Truro, UK
Enrico	Lunghi	enrico.arti@gmail.com	Chinese Academy of Sciences, Peking, China
Petros	Lymberakis	lyberis@nhmc.uoc.gr	Natural History Museum of Crete – University of Crete, Heraklion, Crete
Zdenek	Macat	macat@nppodyji.cz	National Park Podyjí, Znojmo, Czech Republic
Iris	Madge Pimentel	iris.madge@t-online.de	Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany
Michele	Maglia	michele.maglia01@universitadipavia.it	Università degli Studi di Pavia, Italy
Raoul	Manenti	raoulmanenti@gmail.com	Università degli Studi di Milano, Italy
Marco	Mangiacotti	marco.mangiacotti@gmail.com	Università degli Studi di Pavia, Italy
Marko	Maricic	marko12maricic@gmail.com	University of Belgrade, Faculty of Biology, Belgrade, Serbia
Daniele	Marini	marinivet@gmail.com	S.H.I. Sezione Lazio & ANVA; Associazione Naturalistica Valle dell'Aniene, Guidonia Montecelio (RM), Italy
Fernando	Martínez-Freiria	fmartinez-freiria@cibio.up.pt	CIBIO/InBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão; Universidade do Porto, Portugal
Iñigo	Martínez-Solano	inigomsolano@mncn.csic.es	Museo Nacional de Ciencias Naturales, CSIC, Barcelona, Spain
Denoël	Mathieu	mathieu.denoel@uliege.be	University of Liège, Belgium
Glib	Mazepa	glib.mazepa@unil.ch	University of Lausanne, Switzerland
John	Measey	johnmeasey@gmail.com	Stellenbosch University, Stellenbosch, South Africa
Konrad	Mebert	konradmebertmail.com	Global Biology, Birr, Switzerland
Andrea	Melotto	mel8@hotmail.it	Università degli Studi di Milano, Italy
Marcello	Mezzasalma	m.mezzasalma@gmail.com	Department of Life Sciences, Natural History Museum, London, UK; Dipartimento di Biologia, Università di Napoli Federico II, Napoli, Italy
Valentin	Mingo	ValentinMingo@eurofins.com	Eurofins Agrosience Ecotox GmbH, Germany
Marko	Mirč	marko.mirc@ibiss.bg.ac.rs	Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia
Edvárd	Mizsel	edvardmizsei@gmail.com	Hungarian Academy of Sciences, Centre for Ecological Research, Debrecen, Hungary
Gianpaolo	Montinaro	gianpaolo.montinaro@rifcon.de	Rifcon GmbH, Hirschberg, Germany
Martina	Muraro	Marti.muraro93@gmail.com	Università degli Studi di Milano, Italy
Jason	Nagro	jnagro7@gmail.com	Archipelagos Institute of Marine Conservation, Samos, Greece
Dario	Nania	s6danani@uni-bonn.de	Zoologische Forschungsmuseum Alexander Koenig, Bonn, Germany
Annamaria	Nistri	annamaria.nistri@unifi.it	Museo di Storia Naturale dell'Università di Firenze, Firenze, Italy
Nicola	Novarini	nicola.novarini@fmcvenezia.it	Museum of Natural History / Fondazione Musei Civici di Venezia, Venezia, Italy
Christopher David	O'Brien	highland.newt@gmail.com	Highland Biological Recording Group, Inverness, UK
Fabrizio	Oneto	oneto.fabrizio@alice.it	University of Genoa, Italy
Francesco	Origgi	francesco.origgi@vetsuisse.unibe.ch	FIWI-University of Bern, Switzerland

Manuel	Ortiz-Santaliestra	manuele.ortiz@uclm.es	University of Castilla-La Mancha, Spain
Oleksandra	Oskyrko	sashaoskirko@gmail.com	Educational and Scientific Center «Institute of Biology and Medicine», Taras Shevchenko National University of Kyiv, Kiev, Ukraine
Nurhayat	Özdemir	nurhayat.ozdemir@erdogan.edu.tr	Recep Tayyip Erdogan University, Rize, Turkey
Maciej	Pabijan	maciej.pabijan@uj.edu.pl	Jagiellonian University, Krakow, Poland
Emilio	Padoa-Schioppa	emilio.padoaschioppa@unimib.it	Università degli Studi di Milano Bicocca, Italy
Frank	Pasmans	frank.pasmans@ugent.be	Ghent University, Belgium
Mélissa	Peignier	melissapeignier@hotmail.fr	Messerli Research Institute, Vetmeduni Vienna, Medical University of Vienna, Austria
Anja	Pekolj	anjapekolj@gmail.com	Herpetological Society of Slovenia and University of Ljubljana, Biotechnical Faculty, Slovenia
Daniele	Pellitteri-Rosa	daniele.pellitterirosa@unipv.it	University of Pavia, Italy
Daisy	Pensotti	daisy.pensotti@studenti.unimi.it	Università degli Studi di Milano, Italy
Stefano	Pezzi	stefano.pezzi95@gmail.com	University of Antwerp, Belgium
Wagner	Philipp	wagner@allwetterzoo.de	Allwetterzoo Münster, Germany
Orfeo Lucio Antonio	Picariello	picariello@unina.it	Università di Napoli Federico II, Italy
Matteo	Pili	matteo.pili@edu.unito.it	Univeristà degli studi di Torino, Italy
Fabien	Pille	fabien.pille@uliege.be	Université de Liege, Belgium
Cristian	Pizzigalli	pizzigalli.cristian@gmail.com	CIBIO/InBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal
Katja	Poboljšaj	katja.poboljsaj@ckff.si	Center za kartografijo favne in flore, Ljubljana, Slovenia
Lidija	Polović	lidijapolo@t-com.me	The Natural History Museum of Montenegro, Montenegro
Nikolay	Poyarkov	n.poyarkov@gmail.com	Biological Faculty, Lomonosov Moscow State University, Moscow, Russia
Kathleen	Preißler	kathleen.preissler@uni-leipzig.de	Institute of Biology, University Leipzig, Germany
Mihails	Pupins	mihails.pupins@gmail.com	Daugavpils University, Latvija
Eleonora	Pustovalova	minihobbit29@gmail.com	Department of Zoology and Animal Ecology, V. N. Karazin Kharkiv National University, Kharkiv, Ukraine
Lois	Rancilhac	loisrancilhac@gmail.com	Zoological Institute, Technische Universität Braunschweig, Braunschweig, Germany
Catarina	Rato	catarina.rato@cibio.up.pt	CIBIO/InBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal
Edoardo	Razzetti	edoardo.razzetti@unipv.it	Università degli Studi di Pavia, Italy
Pablo	Recio	93.pablo.recio@gmail.com	Museo Nacional de Ciencias Naturales, Madrid, Spain
Ruth Anastasia	Regnet	regnet_ruth@hotmail.com	Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany
Timm	Reinhardt	timmm.reinhardt@gmail.com	Federal Agency for Nature Conservation, Germany
Eva	Ringler	eva.ringler@vetmeduni.ac.at	University of Veterinary Medicine, Vienna, Austria
Max	Ringler	max.ringler@univie.ac.at	University of Vienna, Austria
Dennis	RödDer	d.roedder@leibniz-zfmk.de	Zoological Research Museum Alexander Koenig, Bonn, Germany
Camilo	Rodríguez López	camilo.rodriguez@univie.ac.at	University of Vienna, Austria
Gonzalo	Rodríguez-Ruiz	gmrodriguezruiz@gmail.com	Museo Nacional de Ciencias Naturales – CSIC, Madrid, Spain

Samuele	Romagnoli	Samuele.romagnoli@studenti.unimi.it	Università degli Studi di Milano, Italy
Luca	Roner	lucaroner@gmail.com	MUSE, Trento, Italy
Giacomo	Rosa	giacomorosa@live.it	University of Genova, Italy
Lieze	Rouffaer	lieze.rouffaer@ugent.be	Ghent University, Belgium
Roman	Rozínek	roman.rozinek@naturaservis.net	NaturaServis s.r.o., Hradec Králové, Czech Republic
Maravillas	Ruiz Minano	maravillas.ruiz_minano@biol.lu.se	University of Tasmania, Australia/Lund University, Sweden
Matteo	Ruocco	ruocco@dream-italia.it	Parco Nazionale Foreste Casentinesi, Monte Falterona and Campigna, Italy
Katharina	Ruthsatz	k.ruthsatz@web.de	University of Hamburg, Germany
Enerit	Saçdanaku	eneriti@gmail.com	University of Tirana, Republic of Albania
Roberto	Sacchi	roberto.sacchi@unipv.it	Università degli Studi di Pavia, Italy
Daniele	Salvi	danielesalvi.bio@gmail.com	University of L'Aquila, Italy
Sebastiano	Salvidio	salvidio@dipteris.unige.it	Università degli Studi di Genova, Italy
Mohamed Amine	Samlali	mohamedamine.samlali@gmail.com	University of Marrakech Cadi Ayyad, Faculty of Sciences Semailia, Morocco.
Giovanni	Sanna	giovanni.sanna3@studio.unibo.it	University of Bologna, Italy
Barbara	Santos	barbarasantosbio@gmail.com	CIBIO/InBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal
Marco	Sassoè	marco.sassoe@unito.it	University of Torino, Italy
Lara	Scali		Università degli Studi di Milano, Italy
Stefano	Scali	stefano.scali@comune.milano.it	Museo Civico di Storia Naturale di Milano, Italy
Dino	Scaravelli	dino.scaravelli@unibo.it	University of Bologna, Italy
Mark	Scherz	Mark.scherz@gmail.com	Technische Universität Braunschweig, Germany
Benedikt	Schmidt	benedikt.schmidt@ieu.uzh.ch	Info fauna karch, Neuchatel, Switzerland & Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland
Katja	Schmoelz	katja.schmoelz@hotmail.com	University of Vienna, Vienna, Austria and Eurac Research – Institute of Alpine Environment, Bolzano, Italy
Lisa Maria	Schulte	schulte@bio.uni-frankfurt.de	Department of Wildlife-/Zoo-Animal-Biology and Systematics, Goethe-Universität, Frankfurt/M, Germany
Silke	Schweiger	silke.schweiger@nhm-wien.ac.at	Natural History Museum Vienna, Austria
Sebastian	Steinfartz	steinfartz@uni-leipzig.de	University of Leipzig, Germany
Nina	Serén	ninagseren@gmail.com	1CIBIO/InBIO, Research Center in Biodiversity and Genetic Resources, Vairão; University of Porto, Portugal
Ana	Serra Silva	a.da-silva@nhm.ac.uk	The Natural History Museum, London; University of Bristol, UK
Giulia	Simbula	giulia.simbula@uniroma3.it	Università Roma Tre, Roma, Italy
Tomasz	Skawiński	tomasz.skawinski@uwr.edu.pl	University of Wrocław, Poland
Abderrahim	S'khifa	abderrahim.skhifa@edu.uca.ac.ma	University of Marrakech Cadi Ayyad, Faculty of Sciences Semailia; Morocco
Radovan	Smolinsky	radovan.smolinsky@gmail.com	Faculty of Education Masaryk University, Czech Republic
Mirco	Solé	msole@uesc.br	Universidade Estadual de Santa Cruz, Brasil
Evgeniya	Solovyeva	anolis@yandex.ru	The Moscow State University Zoological Museum M.V. Lomonosov, Russia
Konstantinos	Sotiropoulos	ksotirop@uoi.gr	University of Ioannina, Greece

Jeroen	Speybroeck	jeroen.speybroeck@inbo.be	Research Institute for Nature and Forest (INBO), Brussels, Belgium
Annemarieke	Spitzen – Van Der Sluijs	a.spitzen@ravon.nl	Reptile, Amphibian and Fish Conservation the Netherlands (RAVON), the Netherlands
Manuela	Springolo		Università degli Studi di Milano, Italy
Tariq	Stark	t.stark@ravon.nl	Reptile Amphibian and Fish Conservation Netherlands (Nijmegen), the Netherlands
Susanne	Stückler	susi.stueckler@gmail.com	Natural History Museum, Vienna, Austria
Ursenbacher	Sylvain	s.ursenbacher@unibas.ch	Info fauna – karch, Switzerland
Márton	Szabolcs	szabolcs.marci@gmail.com	Hungarian Academy of Sciences, Centre for Ecological Research, Debrecen, Hungary
Mateusz	Talanda	m.talanda@biol.uw.edu.pl	University of Warsaw, Faculty of Biology, Poland
Alexandra	Telea	alexandra.telea@gmail.com	Faculty of Agricultural and Natural Science, “Ovidius” University of Constanța, Romania
Matthews	Thalassa	tmatthews.matthews@gmail.com	Centre of Excellence for Palaeontology, Iziko Museums of South Africa, Cape Town, South Africa
Nataša	Tomašević Kolarov	natasha@ibiss.bg.ac.rs	Institute for Biological Research “Siniša Stanković”, University of Belgrade, Belgrade, Serbia
Ljiljana	Tomović	lili@bio.bg.ac.rs	University of Belgrade, Faculty of Biology, Belgrade, Serbia
Chiara	Trabella	chiara.trabella@unipv.it	Università degli Studi di Pavia, Italy
Matteo	Trenti	matteo.trenti01@universitadipavia.it	Università di Pavia/MUSE (Museo delle Scienze), Italy
Evan	Twomey	evan.twomey@gmail.com	Vrije Universiteit Brussel, Brussels, Belgium
Peter	Uetz	uetz@vcu.edu	Center for Biological Data Science, Virginia Commonwealth University, Richmond, VA, USA
Siarhei	Uhlianets	s.uglyanets@gmail.com	State Scientific and Production Amalgamation «Scientific and Practical Center for Bioresources» of the National Academy of Sciences of Belarus, Belarus
Marcel	Uhrin	marcel.uhrin@gmail.com	Department of Zoology, Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik University, Košice, Slovakia
Aleksandar	Uroše Chiacchio ić	aurosevic@ibiss.bg.ac.rs	Institute for Biological Research „Siniša Stanković“, University of Belgrade, Serbia
Jean-Pierre	Vacher	jpvacher@gmail.com	Association BUFO, Strasbourg, France
Emiliya	Vacheva	emilia.vacheva@gmail.com	National Museum of Natural History, Bulgarian Academy of Sciences, Sofia, Bulgaria
Mona	Van Schingen	mona.van.schingen@bfn.de	Federal Agency for Nature Conservation, Germany
Isolde	Van Riemsdijk	isolde.vanriemsdijk@naturalis.nl	Naturalis Biodiversity Centre, Leiden, the Netherlands
Mojca	Vek	vek.mojca@gmail.com	Biotechnical Faculty, University of Ljubljana, Slovenia
Guillermo	Velo-Antón	guillermo.velo@gmail.com	CIBIO/InBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal
Ashwini	Venkatanarayana Mohan	ashwinivm30@gmail.com	Technische Universität Braunschweig, Germany
Valentina	Ventimiglia	valentina.ventimiglia@studenti.unimi.it	Università degli Studi di Milano, Italy
Joana	Veríssimo	j_verissimo@cibio.up.pt	CIBIO/InBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão; Universidade do Porto, Portugal
Clement	Vic	vicclement@hotmail.de	Zoological Research Museum Alexander Koenig, Bonn, Germany
Claudia	Viganò	claudia.vigano@studenti.unimi.it	Università degli Studi di Milano, Italy

Leonardo	Vignoli	leonardo.vignoli@uniroma3.it	Università Roma Tre, Roma, Italy
Mireia	Vila-Escalé	vilaem@diba.cat	Diputació de Barcelona, Spain
Andrea	Villa	a.villa@unito.it	Bayerische Staatssammlung für Paläontologie und Geologie, München, Germany
Dani	Villero	dani.villero@ctfc.cat	Forest Science and Technology Centre of Catalonia, Solsona, Spain
Giovanni	Vimercati	gvimercati@outlook.com	Department of Biology, University of Fribourg, Fribourg, Switzerland
Lodovica	Vinci	lodovica.vinci@studenti.unimi.it	Università degli Studi di Milano, Italy
Magda	Vodrážková	mairu@seznam.cz	University of South Bohemia, Czech Republic
Jiří	Vojar	vojar@fzp.czu.cz	Czech University of Life Sciences in Prague, Faculty of Environmental Sciences, Czech Republic
Judit	Vörös	jvoros32@gmail.com	Hungarian Natural History Museum, Budapest, Hungary
Tijana	Vučić	tijana.vucic@bio.bg.ac.rs	University of Belgrade, Faculty of Biology, Belgrade, Serbia
Tanja	Vukov	tvukov@gmail.com	University of Belgrade, Institute for Biological Research, Belgrade, Serbia
Tobias	Uller	tobias.uller@biol.lu.se	Lund University, Lund, Sweden
Kathleen	Webster	kcw49@cornell.edu	Ludwig-Maximilians-Universität München, Germany
Lukardis	Wencker	lwencker@unito.it	Università di Torino, Italy
Ben	Wielstra	ben.wielstra@naturalis.nl	Leiden University, the Netherlands
John	Wilkinson	john.wilkinson@arc-trust.org	Amphibian and Reptile Conservation Trust, Bournemouth, UK
Alexey	Yanchukov	yawa33@gmail.com	Zonguldak Bülent Ecevit University, Zonguldak, Turkey
Volha	Yanchurevich	oyanch@mail.ru	Janka Kupala State University of Grodno, Belarus
Weizhao	Yang	weizhao.yang@biol.lu.se	Lund University, Lund, Sweden
Claus	Zacho	czacho@bio.ku.dk	University of Copenhagen, Denmark
Oleksand	Zinenko	oleksandrzinenko@gmail.com	V. N. Karazin Kharkiv National University, Kharkiv, Ukraine
Ronald	Zollinger	r.zollinger@ravon.nl	Reptile Amphibian and Fish Conservation Netherlands (RAVON), the Netherlands
Marco Alberto Luca	Zuffi	marco.zuffi@unipi.it	University of Pisa, Italy
Valerie	Zwahlen	v.zwahlen@unibas.ch	University of Basel, Switzerland
Annamarija	Žagar	anamarija.zagar@nib.si	Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia

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## Congress venues:

The easiest way to reach the congress venue is via the Metro line 2 (green line, Lambrate station; approx. 5 min through Via Valvassori-Peroni).

There are many restaurants and pubs nearby the congress venue...



**Congress building**  
 Building entrances: via Clericetti 15 or via Valvassori-Peroni 21

**How to reach:**  
 Lambrate railway station  
 Metro (Lambrate station, green line)  
 Tram (lines 19, 33)  
 Bus (lines 45, 175)  
 (+ 5-15 minute walk)

**Botanic garden**

**Where to forage:**  
 1 Congress dining hall ( max ~ 120 people)

**Restaurants and pubs**  
 2 3 5 9 12 13 15

**Take-away**  
 2 4 6 9 11 14 16 17

**In case of beer**  
 3 7 8 9 10

Here you can see the entrance of the congress venue.



view: via Clericetti 15 entrance  
 (other entrance on via Valvassori-Peroni 21)

### Terrace:

Congress dining hall, room 500, room 502

### First floor:

Library, room 503

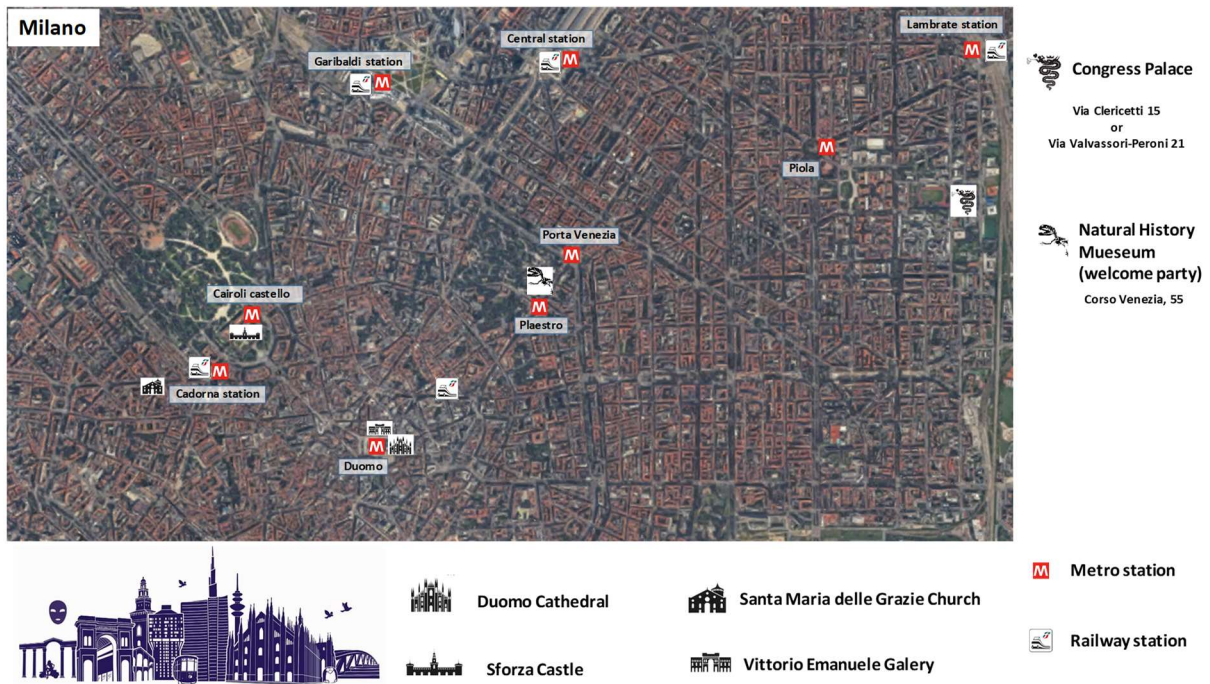
### Ground floor:

Levi room

### Underground floor:

Poster hall

Here you can see the location of some of the main interest points in Milan. The welcome party will be at the Natural History Museum (Metro station: Palestro).





**SOCIETAS EUROPAEA HERPETOLOGICA**

**Agenda – Ordinary General Meeting**

**20<sup>th</sup> European Congress of Herpetology**

**Milano, 5<sup>st</sup> September 2019 – 16.30 – 18.30 (Aula)**



1. Foreword of the President
2. Report of the Treasurer
3. Report of the Auditors
4. Designation of new Auditors
5. Report of the Editors of Amphibia-Reptilia
6. Report of the Editor of Herpetology Notes
7. Report of the Mapping Committee
8. Confirmation of Chair and Report of the Conservation Committee
9. Confirmation of Chair and Report of the Taxonomic committee
10. Report of the Webmaster
11. Changes of Statutes and By-Laws
12. Changes of fees
13. Election of new Council Members
14. Varia

Agenda prepared by Mathieu Denoël,

SEH President



## **SOCIETAS EUROPAEA HERPETOLOGICA (SEH)**

### **Structure of the Society (August 2019)**

#### **Council**

President: Mathieu Denoël (University of Liège – FNRS, Belgium)

Vice-President: Antigoni Kaliontzopoulou (CIBIO, Portugal)

General Secretary: Dan Cogalniceanu (University Ovidius Constanta, Romania)

Vice-Secretary: Francesco Ficetola (Università degli Studi di Milano, Italy)

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Second Co-Editor: Judit Vörös (Hungarian Natural History Museum, Hungary)

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Mapping Committee: Nefta Sillero

Taxonomic Committee: Jeroen Speybroeck & Melita Vamberger

#### **Webmaster**

Edoardo Razzetti

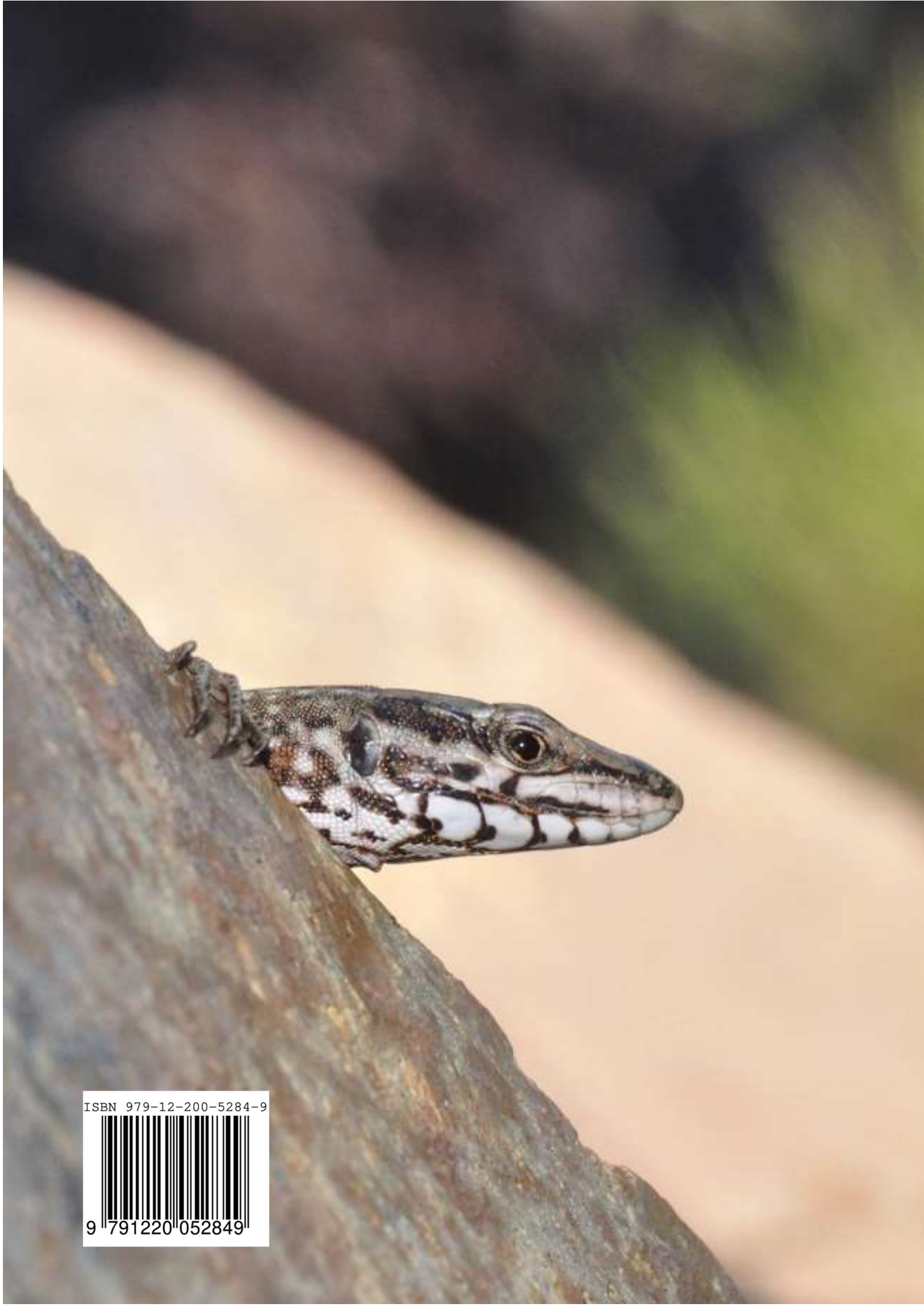
#### **Herpetology Notes**

Hinrich Kaiser (Editor-in-Chief), Anamarija Zagar & Hendrik Müller (Managing Editors), Bina Perl (Layout Editor)

#### **Board of Associate Editors for Amphibia-Reptilia and Section of Editors for Herpetology Notes**

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