

SEH2025

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Oral & Poster presentations



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Foreword

As the organizer of the 2025 Societas Europaea Herpetologica Congress in Bonn and SEH treasurer, it is a pleasure to reflect on this extraordinary event. We welcomed 340 participants—including organizers and volunteers—from 40 countries spanning six continents, demonstrating the truly global and inclusive spirit of our society. The presence of colleagues from almost every European country, as well as from Australia, Brazil, India, Japan, the United States, and beyond, highlighted our international reach and collaborative ambition.

The scientific programme was as diverse as our participants, featuring 188 oral presentations across up to five parallel sessions and 95 posters that showcased current research and new directions in herpetology. Plenary lectures, thematic symposia, and engaging workshops encouraged stimulating debate, interdisciplinary learning, and forward-looking perspectives on conservation, taxonomy, phylogenetics, and citizen science.

A true highlight of our congress was the quartet of plenary talks, each bursting with energy and fresh perspectives. We were captivated by exciting new insights into amphibian chemical communication, inspired by thought-provoking discussions of evolutionary body size dynamics, fascinated by the cutting-edge research on speciation and species boundaries in Caucasian rock lizards, and delighted by a deep dive into the astonishing anatomical diversity of blind snakes (Scolecopidia). The passion and creativity of our speakers—ranging from established leaders to brilliant early-career scientists—sparked lively dialogue and set an inspiring tone for the entire meeting.

Supporting the next generation of herpetologists is a core value of SEH. I am delighted that 128 students took part in the congress, with 17 benefiting from DGHT Student Conservation Grants that eased the path to active participation. Social events, from the opening reception to field trip and museum tours, fostered vibrant exchange and lasting connections.

Organizing SEH 2025 was both a challenge and a privilege. My heartfelt thanks go to the scientific and local organizing committees, our generous sponsors, the many volunteers, and—most of all—every participant, whose enthusiasm and passion ensured the success of this meeting. This Abstract Book records your contributions and energy, and stands as a testament to the strength, talent, and diversity of our herpetological community.

Claudia Koch

Treasurer, Societas Europaea Herpetologica
on behalf of the SEH 2025 Local Organising Committee

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Plenary talks



MORPHOLOGY

Descriptive anatomy's role in herpetology: a dive into scolecophidian discovery

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Abstract

Descriptive anatomy has long been one of the main fields of herpetology, providing essential data on the systematics, taxonomy, and evolution of amphibians and reptiles. Yet, in recent decades, this field has often been regarded as a merely “classical” discipline, mainly overshadowed by results that encompass molecular approaches. In this plenary, I revisit the critical role of detailed anatomical investigation, using threadsnakes and blindsnakes—one of the most enigmatic group of snakes—as a focal point. I will discuss how both traditional and modern approaches, such as manual dissection and high-resolution imaging, continue to provide valuable insights into snake taxonomy, systematics and evolution. To do so, anatomical data will be presented in the context of why and how descriptive anatomy remains relevant in three major areas: (1) taxonomy and systematics, (2) function and functional inference, and (3) morphological evolution, illustrated through both recent and historical anatomical studies on scolecophidians. Finally, this talk invites the public to reflect on how descriptive anatomy remains a vital and resourceful field in herpetology, with many fascinating questions still waiting to be explored.

EVOLUTION

Why miniaturisation is such a big deal

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Abstract

Miniaturisation—reduction in body size to the point that anatomy, physiology, and/or ecology are remodelled—is a widespread biological phenomenon. Due to its propensity to generate anatomical novelty, miniaturisation has played a key role in the origins of many major tetrapod groups. Reptiles and amphibians are excellent study systems for this process: the smallest tetrapods are frogs, the smallest amniotes are lizards, and miniaturisation has occurred numerous times across amphibians and reptiles.

To gain a holistic understanding of the multifaceted consequences, and widespread repetition, of miniaturisation, we need integrative and multidisciplinary approaches.

One key focus must be alpha taxonomy; a comprehensive understanding of patterns and processes cannot be achieved without understanding how widespread the phenomenon is. Yet, taxonomic underestimation is a frequent correlate of miniaturisation because of (1) often-convergent anatomies that result from miniaturisation, and (2) reduced detectability of smaller organisms, and (3) greater attention having often been paid to larger organisms. Over the last few decades there have been some spectacular discoveries among the world's smallest reptiles and amphibians, which have not only improved our understanding of how widespread the phenomenon is, but also continued to push our understanding of the lower limit of body sizes for their respective groups.

Anatomical work on miniaturised lineages can help us to understand the role of miniaturisation in potentiating evolutionary innovation. We can find empirical evidence for different modes of miniaturisation, such as paedomorphism and proportional dwarfism; and now-classic papers showed that developmental variance is increased in miniaturised organisms, providing substrate for selection towards novel structures and relationships among skeletal elements. In some cases, miniaturised lineages have remarkable modifications, such as in the various lineages of blind snakes, with important implications for our understanding (or lack thereof) of the origins of the derived skull anatomy of snakes.

The next horizon is the genome. Understanding the drivers and consequences of miniaturisation at the genomic level may help us to understand the deterministic and contingent consequences of miniaturisation, and provide a substrate for the integration of insights from other disciplines and approaches. These resources will open new avenues of study to understand why getting small is such a big deal.

ETHOLOGY

Amphibian chemical communication: from pheromones to “socially transferred materials”**Lisa M. Schulte**

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Abstract

Courtship pheromones are important components of successful mating in many animals. In amphibians most known pheromones are proteins which are expressed and secreted from species-specific male breeding glands during the reproductive season. Some frog species develop such glands in places of their body that get into direct contact with the female's nostrils during amplexus. One of the highest expressed genes in these glands code Sodefrin precursor-like Factor (SPF) – a proteinaceous courtship pheromone also known from several urodelian species. The transfer of these proteins through the nostrils to a conspecifics' sensory organ defines them as classical pheromones. However, besides the nasal transfer, many species develop male breeding glands in places that do not get into contact with female sensory organs. Instead, the glandular content is transferred subcutaneously. The most spectacular example can be found in the genus *Plectrohyla* (Hylidae), where the males have elongated teeth and develop swollen upper lips composed of SPF producing glandular tissue. Behavioural observations revealed that males press their upper jaw onto the females' dorsum during amplexus, leaving skin scratches with their teeth, suggesting that SPF is delivered transcutaneous in these frogs. While the delivery via teeth marks is rather rare, there is a vast amount of anuran species that use a different way of transcutaneous delivery: in many species male frogs develop nuptial pads on their hands which are composed of breeding glands and pressed onto the female's body during amplexus. First results show that proteins usually classified as pheromones (such as SPF) are also highly expressed in these nuptial pads, but due to the transfer bypassing the female's sensory organs, they cannot be categorized as pheromones, but as socially transferred materials (STMs). However, the evolutionary relation between pheromones versus STMs, as well as the exact path and activity side of STMs is still unknown.

EVOLUTION

Reinforcement versus gene introgression: speciation and species boundaries in the Caucasian rock lizards**David Tarkhnishvili**

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Abstract

Under a classical allopatric-sympatric speciation model, reinforcement is an essential stage before developing a complete pre-zygotic isolation; however, during some period after establishing secondary contact between once isolated evolutionary lineages, with more or less intensive gene flow. Formation of tension hybrid zones is a potential result of hybridization, preventing the expansion of hybrid genotypes outside the contact zone. However, introgression of genes may substantially affect the evolution of contacting lineages and even modify their adaptive strategies, without assimilation of the gene pools. Caucasian rock lizards, a highly diverse monophyletic genus of non-tropical reptiles, are an excellent model for exploring pathways of speciation, including isolation in once-isolated land masses, expansion along the emerging mountain ranges, secondary contacts, and introgression. The exploration of the evolution of this group also brings us closer to the fundamental question – when the divergence becomes irreversible, i.e., we can fearlessly call them species in a classical sense.

Oral presentations



MORPHOLOGY

Sexually dimorphic glands in Neotropical poison frogs and pheromone production

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Abstract

Chemical communication plays a central role in amphibian reproduction, with multiple macroglands, distributed across different body regions and composed of different gland types (e.g., mucous and serous), contributing to the production of proteinaceous pheromones. Among Neotropical poison frogs (Dendrobatoidea), males exhibit sexually dimorphic skin structures involved in courtship, yet the contribution of different gland types and locations to pheromone production remains largely uncharacterized. In particular, specialized mucous glands (SMGs) in the hand integument express high levels of sodefrin precursor-like factors (SPFs), key amphibian sex pheromones. Four morphologically distinct SMG types have been described in the fingers, but their individual roles in pheromone production remain unclear. Additionally, the function of other macroglands, such as the upper arm swelling known as the black arm gland (BAG), is unknown. To address these gaps, we used histology and RNA sequencing to examine the glandular architecture and gene expression in the fingers and arm integument of *Hyloxalus nexipus*, and the fingers of *Allobates trilineatus*. In *H. nexipus*, we identified two sexually dimorphic macroglands, swollen fingers and BAG, both differentially expressing SPF, suggesting complementary roles in courtship. While the fingers primarily contained SMGs, the BAGs were composed of specialized serous glands (SSGs), demonstrating that serous glands can produce proteinaceous pheromones. In *A. trilineatus*, SPF expression in isolated type I SMGs provides direct evidence that this gland type is responsible for

pheromone production. Given their widespread presence across dendrobatoids, our findings support type I SMGs as an ancestral adaptation important to chemical communication in this clade.

BIOGEOGRAPHY

Beeps in the dark: Unravelling the origins of Midwife Toads (*Alytes obstetricans*) in Great Britain using microsatellites

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Abstract

The midwife toad (*Alytes obstetricans*) has been introduced to Great Britain, potentially at various times over the past century, though the provenance and genetic origins of these introduced populations remain unclear. This study used mitochondrial DNA analysis, specifically targeting cytochrome c oxidase subunit I (COI) and NADH dehydrogenase subunit (NADH) genes and the 16S ribosomal gene, to investigate the geographic origin, genetic diversity, and potential source populations of *A. obstetricans* in Great Britain. Non-invasive buccal swab samples were collected from multiple British populations (COI $n = 11$; NADH $n = 4$; and 16S $n = 10$) and compared against reference sequences from known native-range populations in continental Europe on GenBank. Phylogenetic analyses and haplotype network construction revealed distinct clustering patterns, suggesting two potential introduction events rather than a single-source colonisation. All but one sampled British population had haplotypes that matched a population in St Pierre de la Fage (COI), La Sallete (ND4) and Gan (16S), Southern France. The outlier was Cambridge, which did not have an exact match to any sequenced European populations, but showing closest affinity to populations in Southern France and Northern Spain. This indicates that the introduction of *A. obstetricans* within Great Britain aligns with historical records of introductions, over a century ago. Our results provide the first molecular evidence tracing the likely source regions of *A. obstetricans* introductions in Britain and underscore the importance of genetic tools in reconstructing invasion pathways. These findings have implications for understanding the ecological impact of non-native amphibians and raises questions about the potential for the British populations to act as an ark population, aiding in conservation of *A. obstetricans* throughout their native range.

CONSERVATION

Predictors of threat status in the most speciose orders of Amphibians

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Abstract

Amphibians are the most endangered class among vertebrates, so their scientific exploration is crucial for their future conservation. In this study, we investigated how the reproductive strategies and ecological requirements of salamanders & anurans influence their threat status and the trends in population changes. We hypothesised that niche breadth, habitat types, reproductive or developmental mode may influence vulnerability. We collected data on threat status, population trends, habitats, distribution, and reproductive traits (fecundity, egg size, developmental process, parental care) from the IUCN Red List and AmphibiaWeb databases. Phylogenetic comparative methods were employed in the analyses, considering the impact of species relatedness and controlling for it. The results confirmed that, both in Anura & Caudata, generalist species are less vulnerable than habitat specialists and species depositing a large number of eggs are less vulnerable compared to those having smaller clutches. However, results concerning other variables varied between the orders. For instance, salamander species occurring in aquatic habitats or undergoing larval development are less vulnerable than species with direct development. On the other hand, frogs performing longer duration of care and providing more complex protection for their progeny by guarding, internal brooding and viviparity are more threatened. Besides expected similarities, the major differences could be explained by the more complex behaviour and wider distribution of anurans. The strong positive association between threat status and population decline warns of potential positive feedback loops in species decimation, leading to extinction vortices, while the huge portion of data deficient species is also alarming. Our research helps identify the most challenging species and species groups, and ecological or life-history risk factors for conservation purposes.

OTHER

Amphibian diversity in Indonesia: what we know to date and their conservation status

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Abstract

Indonesia is among the most biodiverse regions on earth with a high number of endemism. This holds also true for amphibians. Over the years, more and more amphibian studies were carried out in various regions in Indonesia. Consequently, numerous species have continuously been newly recorded or described, adding significant knowledge to the current diversity of amphibians in Indonesia, especially in the last two decades. The application of advanced methods for biodiversity research and the increasing number of collaborations are considered to influence these efforts in revealing the true diversity of amphibians in the region. Nevertheless, there are no available sources that put together amphibians diversity in Indonesia to date and evaluate its significance for conservation.

Herein we present the most updated checklist for amphibian species of Indonesia by compiling data from various available databases. Additionally, we provide discussion on its significance for conservation and share our perspectives on improving the efforts in understanding amphibian diversity of Indonesia, which could further help to effectively design conservation actions in this biodiverse country.

Ecological correlates suggest non-equilibrium conditions and hybrid zone movement in *Bombina* toads

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Abstract

The fire-bellied toads *Bombina bombina* and *B. variegata* are distributed over distinct areas across Europe. At the interface of their essentially parapatric distribution the species engage in a long and winding contact zone where they also hybridize. I set out to identify environmental parameters underlying the mutual distribution, to reconstruct pattern and process of species' range developments following the Last Glacial Maximum (LGM), and to open research lines for the study of hybrid zone dynamics in a model system. Atlas data reveal that the species' differential ecology resides as much in forestation as in elevation. In two Croatian transects with lowland forestation, hybrid zones are positioned further away from elevated areas than in the absence of lowland forest. The overall species mosaic with isolated mountain strongholds (enclaves) suggests that *B. variegata* was displaced from the surrounding lowlands upon its counterparts' northward advance, following post-LGM climate amelioration. Initial hybrid zone formation was distant from present-day positions. Because lowland forestation constitutes a buffering effect to species replacement, current hybrid zones may not have reached equilibrium conditions, depending on deforestation history and other characters of the landscape. European *Bombina* offers excellent research opportunities on account of i) a variety of hybrid zone configurations ranging from clinal to mosaic, ii) hybrid zones are positioned at the continuous range and at enclaves, and iii) some hybrid zones have stabilized and others are in flux. Future genetic work may shed light on the pattern and process of hybrid zone movement especially at enclaves, although the timing, enclave size and the local environmental signature will affect genetic introgression in ways that may be hard to disentangle. Out of 18 historical *Bombina* studies that were evaluated for promise in the study of hybrid zone dynamics several qualify for extended research.

EVOLUTION

One Shot or Many Chances? Life History Strategies of Caucasian Rock Lizards (*Darevskia*)

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Abstract

We compared life-history traits in two unisexual (*Darevskia dahli*, *Darevskia armeniaca*) and two bisexual (*Darevskia portschinskii*, *Darevskia valentini*) Caucasian rock lizard species to assess how reproductive mode, body size, and environmental conditions shape reproductive strategies. Specifically, we aimed to investigate whether energy allocation into reproduction, egg number and size, and maturation timing differ between unisexual and bisexual lineages, and whether these differences reflect adaptation to habitat stability.

Our results revealed that *D. armeniaca* invests significantly more energy into single reproductive events than its paternal species *D. valentini*, suggesting a strategy suited to less stable environments. In contrast, *D. dahli* does not differ in overall reproductive effort from its paternal species *D. portschinskii*, but lays more, smaller eggs, consistent with an r-selected strategy. In both cases, the parthenogenetic form tends to follow an r-reproductive strategy, typical for lizards occupying less stable environments than their parental species. These patterns highlight how smaller body size and evolutionary divergence modulate the effects of reproductive mode on life-history traits.

Skeletochronological analysis is ongoing. Preliminary data suggest that *D. armeniaca* populations have a higher proportion of younger individuals than *D. valentini*, possibly indicating earlier maturation in the unisexual species as an adaptation to environmental instability. Age structure data for *D. dahli* and *D. portschinskii* are still being collected.

Together, our findings suggest that unisexual *Darevskia* species employ distinct strategies to optimize reproductive success under varying ecological pressures, some maximizing immediate output, others increasing juvenile numbers to buffer against uncertain conditions.

SYSTEMATICS

Molecular Phylogenetics, Biogeography, and Patterns of Limb Loss in the African Skink Genus *Scelotes*

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Abstract

Limb reduction has evolved many times across the squamate tree of life and has been particularly prevalent in the Family Scincidae. Within Scincidae several genera, including *Scelotes*, include species that are limbed, limb reduced, and limbless. *Scelotes* are fossorial and semi-fossorial skinks distributed primarily across southern Africa. Although the genus provides an excellent opportunity to investigate patterns of limb loss within a group of closely related species, to date there have been no genus-wide phylogenetic studies. We provide a divergence dating phylogenetic analysis of *Scelotes* incorporating 18 of 22 recognized species and conduct ancestral state reconstruction to examine patterns of limb loss across the genus. We find that eastern and western species are not reciprocally monophyletic and that diversification within major clades may be related to complex geomorphological and climate changes that occurred in southern Africa, primarily in the Miocene. Ancestral state reconstructions suggest that there were multiple independent instances of limb loss in the genus, and potential limb gain, although there are caveats associated with this result, including difficulties capturing developmental complexities underlying trait evolution in model parameters.

CONSERVATION

Unveiling the Diet of the Critically Endangered Aeolian Lizard *Podarcis raffonei* through an Integrative Approach

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Abstract

Diet is a key ecological trait that significantly influences population dynamics and species interactions. The Aeolian endemic wall lizard, *Podarcis raffonei*, currently survives on only three small islets and a narrow promontory (Capo Grosso). In all these sites trophic resources can be very limited, especially during the dry season and on the promontory where an increasing rat population further impact resource availability. Understanding the diet of *P. raffonei* populations is therefore crucial for monitoring their population trends and identifying potential limiting ecological factors. In this study, we applied an integrative approach combining DNA metabarcoding and morphological analysis of fecal pellets from both *P. raffonei* and rats to develop a protocol for a comprehensive dietary assessment. Fecal samples were collected from three *P. raffonei* populations and the rat population at Capo Grosso. Samples were analysed using two mitochondrial markers (cox1 and 12S) and one chloroplast marker (trnL), along with stereoscopic examination to identify dietary items morphologically. Our results indicate that *P. raffonei* primarily consume arthropods (65.7%), but also ingest a substantial proportion of plant material (34.3%). DNA metabarcoding provided high taxonomic resolution: 91% of arthropod OTUs were identified to species level and 100% to genus level; for plant material, 23% were identified to genus and 84% to family level. Among the studied populations, lizards from Capo Grosso exhibited the narrowest animal dietary niche and showed marked overlap with rats in plant

consumption. These findings offer valuable preliminary insights into the trophic ecology of *P. raffonei* within its unique and isolated habitats. The established protocol will contribute to ongoing and future dietary monitoring efforts as part of the Life EOLIZARD project (Project 101114121 LIFE22-NAT-IT-LIFE EOLIZARD).

ECOLOGY

A bite in a summer night: nocturnal feeding of dice snakes on the shore of Prespa lake

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Abstract

During long-term ecological monitoring of dice snakes (*Natrix tessellata*) on Golem Grad Island—a strictly protected site in Lake Prespa, North Macedonia—we consistently observed nocturnal activity, particularly during summer months. Over 25 night surveys (from 2009 to 2024) resulted in the capture of 368 individuals. Observed night activities, such as swimming and foraging, in July and August (~between 21:00 and 23:00), suggest a partial shift to nocturnal activity, likely to reduce heat stress, dehydration, and predation risk. During one session in July 2024, coinciding with the spawning of the endemic Prespa bleak (*Alburnus belvica*), 28 snakes were captured in just 30 minutes along a five-meter shoreline stretch. Of these, 23 were females—including five gravid—and five were males. A total of 18 fish, averaging 10 cm in length

and 9 g in mass, were regurgitated. Notably, four gravid females had recently fed, challenging the assumption that gravid snakes cease feeding. The presence of exhausted fish in shoreline spawning pools likely provides valuable foraging opportunities. These observations underscore the ecological link between dice snakes and Lake Prespa's endemic fish, both of which face increasing threats from human activity. Poaching is a major concern, as illegal nets often trap and drown snakes, including gravid females, causing severe population decline. Our data show a steady drop from a population once estimated at over 10,000 individuals. These findings highlight the urgent need for stronger enforcement of protective measures on and around Golem Grad Island. Nocturnal activity has been documented in *Natrix* snakes, but most reports are based on citizen science rather than systematic research, leaving this aspect of their behavior understudied.

CONSERVATION

Demography differs between declining and healthy common toad (*Bufo bufo*) populations

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Abstract

During the current biodiversity crisis, amphibians experience some of the steepest population declines. While research often focuses on rare and threatened species, common and widespread species are often neglected. However, even with proportionally minor declines, the loss of common species can have major impacts on ecosystem functions. The common toad (*Bufo bufo*) is a widespread species in Europe and plays a vital role in the energy transfer between aquatic and terrestrial ecosystems. Declines of common toads have been observed in multiple European countries (Belgium, UK, Italy, Switzerland and the Netherlands) and remain largely enigmatic. Putative drivers (climate change, disease, habitat loss, agricultural contaminants ...) may act in concert and can have different effects on the various life stages of the common toad. Therefore, studies on population demography are important to gain insights into population dynamics and may contribute to identifying

life-stage-specific stressors. In this study, we investigate the links between common toad population status (declining vs stable), demography (growth rate and age) and stress levels. We collected samples during spring migration from 10 declining and 10 stable populations across Flanders (Belgium). Dead individuals were used for demographic analysis using skeletochronology (from large bones), while relative telomere length (RTL) as a proxy for stress was determined in buccal swabs. Initial results suggest that declining populations are composed of younger individuals, indicating lower survival rates compared to stable populations. RTL yielded no indications of increased stress levels in declining populations. Final results will be presented during the conference.

The project (Ossobufo) is supported by the Special Research Fund of Ghent University grant number BOF23/GOA/008.

ECOLOGY

Choosing and Using: Microhabitat preferences of poison dart frogs in the lowland Chocó, western Ecuador

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Abstract

Spatial and reproductive ecology of poison frogs in tropical rainforests is shaped by the availability and structure of microhabitats, which offer essential resources such as prey, refuge, and aquatic deposition sites and can be a buffer to extreme weather events. However, microhabitat choice remains poorly understood. This also concerns the occurrences in secondary forests. We used frog activity as a proxy of microhabitat use and asked if and how activity is associated with microhabitat characteristics in secondary forests. In particular, we investigated habitat use in *Epipedobates boulengeri* and *Oophaga sylvatica*. We studied the frogs at 16 different sites from March to May 2023. At each site, we automatically recorded advertisement calls throughout the day to quantify frog activity. In order to characterize the microhabitats, we measured temperature, leaf litter cover, canopy closure, diameter of trees, amount and state of deadwood. We detected various differences in activity pattern and microhabitat preferences between both species. While *O. sylvatica* showed no clear habitat preferences, *E. boulengeri* exhibited specific microhabitat requirements. These were a high amount of decayed deadwood, extensive leaf litter cover, and trees with an average diameter of 90 cm, with a predominantly closed canopy. Our results are in line with other observations that the two poison dart frog species use different parts of regenerating forests.

CONSERVATION

Limiting Habitat Factors for Natterjack Toads (*Epidalea calamita*) in Central German Lignite Mines: Implications for Conservation in Post-Mining Landscapes

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Abstract

Post-mining landscapes in Central Europe offer critical refuge for pioneer species of high conservation concern such as natterjack toads. However, we lack a comprehensive understanding of limiting habitat factors in this particular environment to inform management beyond the coal phase-out. We addressed this knowledge gap through a multi-faceted investigation in two lignite mining areas in Central Germany. First, we examined the genetic structure of local *E. calamita* populations in the face of hybridization with *Bufotes viridis*. Based on microsatellite analyses, we found a notable proportion of hybrids, which — if left unrecognized — can inflate estimates of inbreeding and mislead conservation assessments. Second, we assessed pond-level drivers of breeding success in *E. calamita* across 73 water bodies over three years. We found that precipitation, pond hydroperiod, and breeding site identity were key predictors of high breeding success, while common pond construction parameters such as depth and surface area were more relevant for general amphibian species richness. Moreover, water acidity was a critical parameter for all amphibians. Thus, pond construction programs that focus on enhancing diversity may negatively affect specialist species like *E. calamita*. Third, we investigated whether the terrestrial microhabitat use of juvenile and (sub)adult toads differs using 80 microhabitat plots equipped with artificial coverboards and detection dog transects. We found pronounced life stage-specific differences in habitat selection: juveniles preferred flat areas near ponds, while (sub)adults used steeper microhabitats further away. Occupancy rates and persistence were higher in an unreclaimed area compared to a structurally homogenized, reclaimed site. Together, these findings illustrate that successful conservation of *E. calamita* in post-mining environments requires targeted, species- and life stage-specific habitat management.

CONSERVATION

Are Frog Populations at Risk? Regional PVA of the *Pelophylax esculentus* complex in fragmented habitats of South Banat shows low extinction risk

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Abstract

This study evaluates the spatial structure and viability of the *Pelophylax esculentus* complex metapopulation system in South Banat, Serbia. A habitat suitability model, based on 10 biotic and abiotic variables, was created to identify source and sink regions in the landscape, with a suitability threshold of $HS=0.8$. The dispersal function was modelled with an average distance of 0.7 km to reflect potential species dispersal within the region. The carrying capacity (K) of suitable regions was calculated using average habitat suitability and the number of suitable cells ($K=AHS \times NOC$). A ceiling growth model was applied, assuming no density dependence below K . The population structure model identified 13 viable regions. We used RAMAS MetaPop to assess extinction risk and minimum viable population size (MVP). The demographic model was simulated for 25% stochasticity to reflect anthropogenic pressure on all demographic parameters, including dispersal. The initial population size ($K/10$) was distributed among age classes 2–6. Simulations were run over 100 years (25–30 generations) with 1,000 iterations. Results showed strong spatial structure and overall stability, with a slight decline ($\sim 5\%$) over time. The local extinction threshold of 10% indicated extinction in 6 of 13 demes in the defined time frame, while the remaining 7 demes exhibited lower extinction risk. Sensitivity analysis showed that increased egg and larval mortality, along with reduced fecundity in reproductive age classes, had the greatest impact on extinction risk. In contrast, a reduction in initial population size had minimal effect, while dispersal stabilised the dynamics. The estimated minimum size of the metapopulation was $\sim 3,095$ individuals, with an adult MVP of 250–300. Continued decline and demographic shifts—particularly in early life stages—could increase the extinction risk to 10%, meeting IUCN Red List criterion E for classification as Vulnerable in regional assessments.

ECOLOGY

Tracing tetrodotoxin distribution and variation in natural populations of Modern European Newts

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Abstract

Tetrodotoxin (TTX) is a potent neurotoxin known mainly from marine organisms. However, it has also been described in three clades of anurans and within caudates, only in newts. Most of the studies of TTX in caudates focused in three species of New World Newts (i.e., *Notophthalmus viridescens*, *Taricha granulosa*, and *T. torosa*), with a few reports on TTX's occurrence in Eurasian Newts. In addition, and despite recent phylogenetic updates, the distribution of this molecule has not been addressed in an evolutionary context. Here, we combined a non-invasive sampling protocol with ultra-sensitive detection analysis to characterize the amount of TTX in different populations of 12 species comprising the six described genera of Modern European Newts. In high agreement with previous reports, our results indicate a marked variation in TTX concentration among and between populations. Although such high variability precludes a definitive conclusion, our results, combined with previous studies, point to a shared capacity for TTX accumulation and secretion by all modern Eurasian and New World newts. In our subsequent studies, we hope to determine whether this trait is shared by all newts, including the so-called "primitive newts" of the genera *Pleurodeles*, *Echinotriton*, and *Tylototriton*.

ECOLOGY

The influence of plant composition on an endangered grassland specialist reptile, the Hungarian meadow viper

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Abstract

The Hungarian meadow viper (*Vipera ursinii rakosiensis*) is one of Hungary's most endangered vertebrates, whose population is not growing significantly despite conservation measures. When designing conservation measures, it is crucial that they are evidence-based and fit to the habitat preferences of the target species. In the case of the grassland specialist meadow viper, vertical vegetation structure already proved to influence habitat selection, but the effect of horizontal vegetation structure and plant species composition has not been well understood yet. In the present study, we used botanists' coenological records to investigate the effects of variables related to the horizontal and functional composition of vegetation on the occupancy and abundance of the Hungarian meadow viper. In a survey period during spring, we collected viper data in 59 sampling quadrats alongside coenological records, then used single-season occupancy models, N-mixture models and performed model selection to investigate the effects of variables on the vipers' occupancy and abundance. The best models included the NMDS1 axis, species richness, graminoid-forb ratio and height of plants as explanatory variables for both occupancy and abundance. Wetter meadows with less plant species, more forb species against graminoids and habitats with lower growing plant species were preferred by the vipers. Our results suggest that the horizontal structure and functional composition of the vegetation influences the habitat selection of vipers, and also draws attention to the threats posed by increasingly frequent dry periods.

ECOLOGY

Ten years of green toad (*Bufo viridis*) population studies in Eastern Austria

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Abstract

Over the past decade we conducted extensive fieldwork and research on European green toads (*Bufo viridis*) in Eastern Austria, with a particular focus on their ecology in anthropogenic environments. This long-term study integrates results from several urban and peri-urban populations in Vienna, and a more natural population in Illmitz, Burgenland. Using capture-mark-recapture (CMR) techniques supported by semi-automated photo identification, we tracked individual toads over multiple years, yielding insights into population size, structure, sex ratios, movement ecology, and reproductive phenology. We observed markedly different population dynamics between populations (and even among subpopulations) that did not follow a consistent urban–rural gradient. Instead, these differences appear to be driven by specific local habitat features (e.g., water availability, land-use intensity, or degree of isolation) rather than by overall urbanization level alone. Our findings underscore the species' capacity to rapidly colonize urban habitats, particularly favoring open, early-successional environments such as construction sites. Nonetheless, particularly urban populations showed reduced movement ranges and hardly any exchange of adult toads between nearby breeding sites, raising questions about their mode of dispersal. As expected, during the breeding season males clustered at and close to the breeding sites, while females were more commonly observed in their wider surroundings, and overall, our analysis indicated a near 1:1 adult sex ratio. Lastly, we found that individual reproductive timing was dependent on the toad's body size and condition. Collectively, the data offer a comprehensive perspective on the demographic and ecological responses of *B. viridis* to urbanization and provide a foundation for conservation strategies in rapidly changing environments.

CONSERVATION

A novel approach to landscape-scale species conservation funded through development impact

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Abstract

Great crested newts (*Triturus cristatus*) are strictly protected under Annex IV of the EC Habitats Directive, and developers must obtain licenses and set out stringent mitigation measures where risk of harm to individual newts is likely. However, an England-wide review demonstrated that the current system caused planning delays and that the huge expense of on-site mitigation showed little evidence of conservation success.

District Licensing was therefore launched in 2018 as a strategic alternative licensing system in partnership with amphibian and freshwater NGOs, focusing on expanding newt populations at the landscape-level rather than at the site-level, whilst speeding up planning. As derogation from statutory legislation, licensing must meet all standard legal tests, including no impact to Favourable Conservation Status.

Region-wide eDNA surveys inform species distribution modelling that maps habitat suitability for great crested newts and identifies strategic areas suitable for expansion of newt populations. The conservation strategy delivers specialist-led, high-quality habitat at a higher ratio than under standard licensing that is legally secured for 25 years and focuses on establishing pond networks to facilitate metapopulation expansion.

Here we present conservation outcomes of the scheme, with initial evaluation suggesting the scheme is working well. The latest monitoring data demonstrate that 84% of mature sites and 70% of mature ponds are occupied by great crested newts, as well as supporting other rare and priority species. Compensation ponds are more than twice as likely to be occupied than the average English pond and suitability for great crested newts has increased at the landscape-scale. Monitoring data also highlight that new ponds can take 4-6 years to be fully colonised, so the delivery of high-quality, well-managed habitat is crucial for the long-term success of great crested newt conservation.

OTHER

Endocrine disruption by microplastics in amphibian larvae: hormonal receptor responses in *Rana* and *Bufo* tadpoles

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Abstract

Microplastic pollution poses a significant threat to aquatic ecosystems, raising concerns about its potential to disrupt endocrine function in amphibians. This study investigated the impact of microplastic ingestion on the expression of key endocrine cells and hormone receptors in tadpoles of three species – *Rana dalmatina*, *Rana temporaria*, and *Bufo bufo* – with 12 individuals per species per Gosner stage. Tadpoles were fed experimental diets containing environmentally relevant concentrations of microplastics over a 21-day period. Specimens were collected at different Gosner developmental stages to evaluate potential variations in hormone-related cellular activity. Longitudinal sections were prepared from the head and abdominal regions to target relevant tissues. Immunohistochemistry was performed to detect the expression of chromogranin A, ghrelin, glucagon, serotonin, somatostatin, and growth hormone secretagogue receptor 1 (GHS-R1). Five representative microscopic fields per group were analyzed. Results showed notably elevated expression of ghrelin and its receptor GHS-R1 in microplastic-exposed tadpoles, suggesting an increase in hunger signaling. The microplastic group displayed a higher average number of ghrelin-positive neuroendocrine cells compared to controls. Meanwhile, chromogranin A, serotonin, somatostatin, and glucagon displayed relatively consistent expression across all groups. The interpretation of these hormonal changes remains challenging due to the lack of existing comparative studies on endocrine responses in larval amphibians. Our findings highlight the importance of early developmental stages in evaluating endocrine disruption. This study provides initial insight into how microplastic exposure may influence neuroendocrine regulation in amphibians and underscores the urgent need for baseline hormonal data to better understand and compare these effects across developmental phases.

CONSERVATION

Heat-lovers going north: monitoring two reptile species at the northern margins of their range

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Abstract

The asp viper (*Vipera aspis*) and the Western green lizard (*Lacerta bilineata*), which typically inhabit dry habitats with plenty of basking opportunities, find themselves in northeastern France at some of the northernmost locations of their range, with populations scattered in fragmented dry and sunny habitats. We have been monitoring these two reptiles in the Grand Est region every three years since 2020, with a dynamic site occupancy model encompassing over 300 sampling units for each species, randomly distributed within their range. We present here the results from the first years of this program, discuss the methodological implications, and provide conservation recommendations. Occupation rate for *L. bilineata* is high overall ($\hat{\theta} = 0.73$) but strongly differs between western and eastern populations within the study area, while occupation rate for *V. aspis* is low overall ($\hat{\theta} = 0.23$). Detection probability is higher for *L. bilineata* ($p = 0.68$) than for *V. aspis*, for which meteorological conditions are quite impactful (highest $p = 0.32$ at 21°C). For the latter, more visits are needed to improve detection, which take longer on average when the species is present. Hedgerows and forest edges consistently stand out as of the utmost importance for both species on occupation prediction.

Global warming could benefit the thermal niche of these species at their northern limit, but their limited dispersal capacity makes any beneficial effect rely on functional ecological networks (maintained or restored), without which the colonisation of new favourable habitats will remain impossible. A few sampling units are located beyond their current range (at higher altitudes or latitudes) in the hope to monitor a potential gradual shift, expected if effective conservation and nature restoration actions are implemented. For example, vineyards can be suitable habitats, but only if they still display hedgerows, dry stone walls or vegetated embankments, and are kept without pesticides.

ECOLOGY

Keystone salamanders: testing the occurrence of keystone niche individuals in salamanders' populations

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Abstract

Intraspecific variation in resource use is a fundamental driver of ecological dynamics, yet the differential contribution of individuals to population niche width is usually neglected. Recent studies introduced the concept of Keystone Niche Individuals (KNIs): individuals which contribute disproportionately to the total niche width (TNW) of their population. Here, we evaluate the occurrence and ecological significance of KNIs in salamander populations.

We used stomach content data of 482 individuals from 21 populations of seven salamander species: *Speleomantes strinatii*, *Speleomantes imperialis*, *Euproctus montanus*, *Salamandra atra atra*, *Salamandra atra aurorae*, *Salamandrina perspicillata*, *Ichthyosaura alpestris*. Individual-level contributions to TNW were quantified by iteratively removing one individual from the population at a time and measuring the resulting changes in TNW. We also assessed two key predictors of individual contribution to TNW: individual niche breadth (as Shannon diversity index) and inter-individual diet variation (Psi).

Our results showed that KNIs are present in all populations studied. Furthermore, KNIs were found to influence niche structure in both directions, promoting both niche expansion and contraction, with individuals contributing to niche broadening as well as driving niche contraction. Finally, both individual niche breadth and individual specialisation significantly contributed to an individual's disproportionate influence on TNW. Individuals with broader dietary niches (higher Shannon values) and greater individual specialization (higher P_{Si} values) were more likely to be identified as KNIs.

TAXONOMY

The Monitor Lizards of Thailand (Varinade: Varanus: Squamata)

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Abstract

The monitors of Thailand have been recognized by the relevant national authorities in Thailand as consisting of four taxa: *Varanus bengalensis nebulosus* or *Varanus nebulosus*, *Varanus dumerilii*, *Varanus rudicollis*, and *Varanus salvator* (Nabhitabhata et al., 2000; Nabhitabhata & Chan-ard, 2005). There have been other taxa in Thailand which have been recorded, with evidence (*Varanus bengalensis bengalensis*), without evidence (*Varanus flavescens*), or have been synonymized (*Varanus salvator komaini*). These are discussed to give more clarification of the valid taxa in Thailand. Geographic distribution and habitats for the known taxa in Thailand are discussed as well as some ecological notes.

CONSERVATION

New solutions for amphibians in gully pots

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Abstract

As RAVON reported in 2012, between 0.5 to 1 million adult amphibians die in gully pots every year, in the Netherlands alone. Newts, salamanders, frogs and toads get washed away into the sewer system. Since 2013 several solutions have been tried out in various towns and cities, but none proved successful: climbing mats and perforated strips never last long and are damaged during annual cleaning schemes, while other solutions impede the drain function.

In controlled field tests conducted in spring 2023 and 2024, over 90 to 95% of the common toads and over 95% of the newts in the test successfully used a new corner profile developed by drainage systems manufacturer TBS-SVA to escape from rectangular gully pots. This is a robust, affordable design that guarantees the draining function of gully pots. RAVON now recommends this corner profile for this type of gully pot.

Meanwhile, the search for solutions for round gully pots and for redirecting water flows in the kerb itself continues. Further product development and tests are being planned for the coming years.

Finding solutions for this unnoticed problem is essential to saving urban amphibian populations. Also in relation to the development of Sustainable Urban Drainage Systems (SUDS) RAVON continues to be involved in amphibian-friendly solutions.

RAVON is currently scaling up the project in alliance with two other organisations. We are planning projects in 40 cities across the country to identify hotspots in close cooperation with toad patrols and other volunteers. The project also includes promotion of garden ponds and adoption campaigns for schools and companies. A video of 60 seconds, starring a toad that uses the amphibian ladder, went viral and got just over 2.5 million Instagram views in the first two months.

CONSERVATION

Dark side of extraction of lithium, boron and arsenic: impact on local amphibians and reptiles

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Abstract

Due to energy crisis, European governments showed increased interest in continent-wide exploitation of lithium as a valuable source. In the process of lithium extraction from the ground, another two chemical elements are released to the environment: boron and arsenic. Being substantially concerned about impacts of such mining projects on local biodiversity, we surveyed literature about the known impact of lithium, boron and arsenic on amphibians and reptiles. We found surprisingly small amount of information (21 and 10 references in total for amphibians and reptiles, respectively). However, these scarce literature data are revealing somewhat disturbing facts: Exposure of amphibians to lithium (10 references found in total), boron (two references in total) or arsenic (9 references found in total), especially in aquatic ecosystems, lead to a wide range of adverse effects, both for individuals at different developmental stages and for whole populations. In some of the species studied, reduced survival of juveniles and reduced fecundity of adults were observed. Current knowledge on the impact of lithium on reptile populations indicates that the release of lithium from soil into the environment leads to the disruption of local populations, and, in the long term, possibly to their extinction (one reference found in total). In snakes and lizards, a negative effect of arsenic accumulation has been observed in phenotypic traits essential for survival; moreover, arsenic accumulated in the bodies of females is transferred to their offspring (9 references found in total). No studies about impact of boron on reptiles were found. In conclusion, the effects of lithium, boron and arsenic extraction from the ground on local amphibian and reptile populations require more thorough research.

OTHER

The venom-delivery system of the non-front fanged snake *Heterodon nasicus*: About venom compositions, gland imaging and ketamine contamination

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Abstract

Snakebites fascinate and worry in equal measure - but behind the bite lies a complex biological system. While most snakes are harmless to humans, little is known about their venom delivery systems, like the the western hognose snake (*Heterodon nasicus*). A snake commonly considered as non-venomous and being a widespread pet. The project aimed to improve our understanding of its venom, biological functions, as well as the potential risks to humans.

For the first time, the venom composition of its two subspecies (*H. n. nasicus* and *H. n. kennerlyi*) were described by proteo-transcriptomics. The snake venomics approach by mass spectrometry (MS) showed a very simple composition. Beside svMP and CRISP, it consists largely of a previously little-known toxin family, sulfhydryl oxidases. Furthermore, we investigated the spatial distribution of its toxins by the first MS imaging of a Duvernoy's venom glands. This method enables the detection of individual toxins within the tissue at micrometer precision.

Additionally, high-resolution MS revealed that substances injected to aid in venom extraction, such as ketamine and pilocarpine, are unexpectedly sequestered into the venom, at concentrations that could potentially exert biological effects. Calling into question if such contaminations in venoms are relevant for toxicology and venom research in the future.

CONSERVATION

Using Computer-assisted Photo-Identification for Squamate population studies

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Abstract

Population monitoring is an essential tool for wildlife conservation. Protocols based on individual identification, such as mark–recapture, allow for the modelling of population sizes and dynamics. In this context, the viability of studies depends on the available technologies for reliable identification of individual animals. Studies focused on squamate populations have used methods such as scale and toe-clipping, heat-branding or internal emitters, which are invasive and can induce stress and pain for the individuals. Photo-identification is another potential method that uses long lasting natural patterns such as spots or scales to recognize individuals without applying a mark or an emitter. However, it can be time-consuming if pictures are analyzed manually. To overcome this, several photo-recognition programs have been developed. But which should you use for your focus species? And what part of the animal should be photographed? To answer these questions, this study tested efficiency of three commonly used programs (I3S Pattern, WildID and HotSpotter) for individual recognition of squamates using pictures of the head, jaws, back, etc. At the time of writing this abstract, species from 24 families spanning the squamate phylogenetic tree have been studied, with more being analyzed. The results will allow to provide recommendations on area of interest to photograph and software choice for the different groups, from geckos to colubrids and more.

CONSERVATION

The marsh frog invasion: diversity, pathways, opportunism and effects

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Abstract

Amphibians are declining worldwide, but some species stand out for their ability to invade large areas after being introduced. While some invasions are well documented, others are more cryptic and often underestimated despite local warnings. This is the case of the marsh frog, *Pelophylax ridibundus* for which an integrative overview of the invasion patterns, ecology and risks to native amphibians is lacking. To fill this gap, we reviewed published invasion cases in Europe and conducted an interdisciplinary study of the phylogeography, spatial, trophic and thermal ecology of the marsh frog (*Pelophylax ridibundus sensu lato*). We found that introductions have occurred in 19 European countries, involving many lineages of the species from three continents, which fits well with the history of importation of live frogs into Europe, mainly for consumption of their legs, but also for research, education and ornamentation of ponds. These introductions resulted in some country-wide invasions, which led to large spatial niche overlaps with native amphibians. The establishment of the invaders was facilitated by their opportunism, characterized by their tolerance to a wide range of temperatures, their presence in various pond environments, and their ability to prey on a large variety of both aquatic and terrestrial organisms, including other amphibians such as newts and tree frogs. Taken together, these results place the marsh frog as one of the most complex and opportunistic invasive amphibian species in the world. They call for more research and genome-wide molecular analyses to assess patterns of genetic diversity, structure and gene flow between the lineages involved across the whole invaded and native areas. They urge the need for a complete commercial ban on the import of live water frogs to prevent further introductions but also limit the risk of multiplying lineage combinations, a Pandora's box that could boost the invasiveness of the species.

ECOLOGY

Multiple paternity in smooth-fronted caimans – ecological and behavioural implications

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Abstract

Over the past ten or more years, studying how different animals mate has changed our understanding of their mating habits and extraparental mating. We have discovered that many animals, including all species of crocodiles and their relatives, frequently produce offspring with multiple fathers. The smooth-fronted caiman (*Paleosuchus trigonatus*) is one of the few remaining species to study in this regard. For our study, we examined the microsatellite DNA of smooth-fronted caimans in French Guiana. This species is very secretive and difficult to observe in the wild. We collected scute samples from three populations and five groups of freshly hatched neonates. We determined the minimum number of fathers for each group of offspring by verifying allelic diversity of all samples with CERVUS and with the parentage reconstruction program GERUD2.0. Our findings show that it is common for these caimans to have multiple fathers. In fact, 60% of the newborn groups we studied had at least two fathers. We discuss what this finding might mean for their behaviour and environmental constraints, and suggest further studies to improve our understanding of this species' mating habits and the environmental challenges it faces.

OTHER

Hybridogenetic Pelophylax esculentus systems in Ukraine: reproduction and maintenance

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Abstract

Water frog *Pelophylax esculentus* complex involves *P. lessonae*, *P. ridibundus*, and di- and triploid hemiclonal hybrid *P. esculentus* (LR, LLR, LRR genotypes). Hybrids transmit clonal parental genomes in gametes and depend reproductively on parental or other hybrid forms providing lacking genomes to restore hybrid genotype. Mixed populations of *P. esculentus* and parental forms with circulating clonal genomes are called population systems. Their sustainability relies on hybrids' gametes (haploid or diploid, clonal or recombinant, or their mixture) and certain genotypes' survival. Thus, beside a model for hemiclonality mechanisms studies, such systems also present a great example of unusual intrapopulation selection mechanisms.

Ukraine hosts most of known types of *P. esculentus* systems: with one parental species (L-E, R-E types), both (L-E-R), often with triploids (-Ep). Siverskyi Donets River basin, largest in the East, is especially rich in R-E systems (e.g. R-E-Ep) and devoid of adult *P. lessonae*. Selective mortality during pre- and postmetamorphic stages impacts the progeny of *P. lessonae*, *P. ridibundus*, and di- and triploid *P. esculentus*, of one or both sexes. The basin hosts systems with single-sex hybrids too. In contrast, basins of the largest West-Center-North rivers (Dnipro, Dniester, Dunai) contain, beside simpler types, also L-E(-Ep)-R systems with probably even more complicated selection patterns balancing multiple forms at once.

Selection within each system presumably depends on features of and interaction between clonal genomes, also possibly on habitat conditions. Even in systems of the same type, patterns of gamete production and genotypes elimination were shown to differ. Maintenance of unisexual hybrid lines may rely on sex-determining factors in transmitted clonal genomes. Rich Ukrainian water frog fauna offers a unique diverse natural model for studying evolution and flexibility of clonal reproduction strategies and selection during ontogenesis.

ECOLOGY

Ontogenetic dietary shifts in an apex predator, the Japanese giant salamander

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Abstract

Species exhibiting gigantism, particularly apex predators, play a crucial role in food webs, shaping ecosystem structure and function. In species with exceptionally broad size ranges, growth may drive ontogenetic dietary shifts (ODSs)—changes in diet across an individual's lifespan—leading to a pronounced increase in trophic position (TP). Larger individuals may gain access to prey higher in the food chain and inaccessible to smaller conspecifics, eventually becoming apex predators. In this study, we investigated ODSs for the first time in one of the world's largest amphibians, the Japanese giant salamander (*Andrias japonicus*), focusing on how changes in prey consumption and trophic position relate to individual body size. We combined stomach content analysis (SCA) and stable isotope analysis (SIA), incorporating isotopic mixing models to assess how key trophic metrics vary with body size. We identified pronounced ODSs: smaller individuals primarily consumed small aquatic insects, whereas larger salamanders predominantly fed on fish, anurans, and freshwater crabs. These dietary shifts were mirrored in a non-linear increase in TP with an inflection point indicating a sharp increase. This transition likely reflects morphological and physiological adaptations associated with gigantism, enabling exploitation of larger prey. Overall, our results suggest that gigantism in predators such as giant salamanders may be adaptive by facilitating access to higher trophic levels through extended growth, ultimately allowing individuals to function as apex predators. These findings underscore the ecological significance of gigantism and

emphasize the importance of conserving this threatened endemic species within its natural habitat.

EVOLUTION

Divergence-dependent processes drive unique yet parallel genomic evolution in island lizards

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Abstract

When the same species colonizes multiple environmental gradients, independent adaptation along similar gradients can involve the same genetic changes. Archipelagos provide a unique opportunity to assess the role of parallel evolution vs. unrelated adaptive changes, because different islands often have been repeatedly colonized by organisms from the mainland, exposing them to novel environmental gradients. The Italian wall lizard, *Podarcis siculus*, reached the Aeolian islands in multiple waves of colonization. These islands expose populations to steep gradients of temperature and productivity. We sampled multiple populations of *P. siculus* from each of the Aeolian islands along these environmental gradients. We measured multiple traits of individuals, and used a dense panel of SNPs (RAD-seq) to characterize their genomic features. A substantial proportion of genomic variation was shaped by colonization history. In each island, selection analysis identified multiple putatively adaptive loci. We found evidence of both parallel evolution and unrelated adaptive changes. Similar patterns of genetic variation along the gradients of multiple islands were found in 149 genes at different levels, from shared single mutations to

distinct mutations in the same gene. Most of the genetic changes were unique to islands and had varying chromosomal distribution, but gene set analysis revealed consistent genetic changes across islands that were associated with overrepresented biological functions. Genetic parallelism was more pronounced at the functional level than at the mutation level, emphasizing repeated selection for similar functional outcomes. Adaptive trajectories were remarkably parallel across islands, more than expected under neutral evolution, especially between islands with limited genetic differentiation. Environmental factors and population divergence influence parallelism, but their effects vary across the diverse facets of parallelism.

EVOLUTION

Genomic divergence and introgression in a broad hybrid zone between two bimodal viviparous salamander subspecies

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Abstract

Hybrid zones offer valuable opportunities to study speciation at varying stages of divergence, particularly when they involve taxa with pronounced differences in phenotypic and reproductive traits. In the Iberian Peninsula, the fire salamander (*Salamandra salamandra*) exhibits remarkable morphological and reproductive diversity. We focused on a fine-scale transect across a contact zone between two subspecies—*S. s. bernardezi* (pueriparous) and *S. s. gallaica* (larviparous)—which differ in reproductive mode and life-history traits. Despite these differences, previous studies have documented extensive gene flow, mitochondrial introgression, and clinal variation in reproductive phenotypes and associated life-history traits across the hybrid zone.

To investigate the genomic landscape of divergence between these subspecies, we used ddRAD-seq to generate genome-wide SNP data. We characterized both genome-wide and locus-specific patterns of divergence and introgression, and applied population genomic and cline analyses to identify putative barrier loci. Our results reveal widespread genomic admixture, alongside a subset of loci showing reduced introgression. We also found substantial variation in the width and position of individual clines. This supports the interpretation of a broad contact zone not maintained by strong genome-wide selection against hybrids. These findings provide insights into the genomic architecture of early-stage speciation and inform ongoing discussions regarding the taxonomic status of *S. s. bernardezi*.

SYSTEMATICS

Hiding behind a giant – Untangling the gemsnakes of the *Liophidium torquatum* complex from Madagascar

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Abstract

Liophidium torquatum (Boulenger, 1888) is a species of gemsnakes (Pseudoxyrhophiidae) endemic to Madagascar, but considered to be widespread over large parts of the island.

New phylogenetic data revealed that this taxon is paraphyletic, with one clade of the *L. torquatum* species complex being more closely related to *Liophidium mayottensis*, an island giant endemic to the Comoran island of Mayotte, than to other populations of the taxon.

We herein demonstrate that *Liophidium torquatum* in its broad current definition needs to be split into three species based on differentiation in (I) multiple mitochondrial and nuclear markers, (II) colour patterns, (III) hemipenial structures, and (IV) distinct distribution ranges of each species.

Thus, we recover the true *L. torquatum* as a monophyletic taxon and will describe two species new to science. We also shed light on the evolutionary history of *L. mayottensis*, proposing its colonization of Mayotte from Madagascar via the South Equatorial Current.

CONSERVATION

Amphibian conservation responses to fish introduction – Workshop introduction

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Abstract

[This abstract is for the introductory presentation in the Workshop “Amphibian conservation responses to fish introduction”.]

The introduction of fish is recognised as a major cause of decline for some amphibians. Despite a growing evidence base on the negative consequences for amphibians, practitioners face uncertainty over the best approaches to preventing fish introduction and responding if fish are introduced. To introduce this workshop, the general issues are discussed using examples from across Europe. The interactive part of the workshop is introduced by asking participants to share their views on a range of topics. These topics include: the effectiveness of strategies for preventing fish introduction, such as pond design and public engagement; the effectiveness of methods for removing fish from amphibian breeding sites; minimising harm arising from fish removal exercises; the costs and legal issues associated with fish prevention and removal exercises; identification of evidence gaps; identification of opportunities to trial new approaches.

EVOLUTION

Target capture sequencing sheds light on hybridogenetic frogs

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Abstract

Frogs of the genus *Pelophylax* exhibit a fascinating mode of reproduction known as hybridogenesis, where hybrids between two species will only transmit the genome of one parent to their offspring. This allows a clonally transmitted genome from a hybridogenetic species to act as a genetic parasite, reproducing itself at the expense of a sexual host species. This extraordinary phenomenon contributes to some *Pelophylax* species being considered amongst most dangerous introduced amphibians within Europe. Previously, the molecular tools available for the study *Pelophylax* have been limited, which is especially problematic because the taxa can be hard to differentiate morphologically. We apply a previously published target capture bait set, FrogCap, to *Pelophylax* samples from the Netherlands and Poland. We show that this method can recover data from thousands of nuclear genes, distinguish between parental species and their hybrids, and reliably identify triploid samples. We also confirm the presence of introduced *Pelophylax* populations within the Dutch coastal dunes and observe a massive amount of mito-nuclear discordance within Dutch *Pelophylax*. Our results show the potential benefits that target capture sequencing brings to the investigation of one the most interesting amphibian systems in Europe.

ECOLOGY

Impact of bisphenol A and ethinyloestradiol on gonadal differentiation and digit ratio of the *Pelophylax ridibundus*

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Abstract

This study investigated the impact of two common environmental endocrine-disrupting compounds (EDCs) — bisphenol A (BPA) and 17 α -ethinylestradiol (EE2) — on gonadal development and the second-to-fourth digit ratio (2D:4D) in *Pelophylax ridibundus*, with 2D:4D proposed as a potential non-invasive biomarker of exposure to EDCs. Tadpoles were raised in outdoor mesocosms and exposed to environmentally relevant concentrations of BPA (10^{-7} , 10^{-8} , 10^{-9} M) and EE2 (10^{-9} M). EE2 exposure significantly skewed the sex ratio toward females, increasing the likelihood of female development, while BPA showed no effect on sex ratios. Moreover, histological analysis identified intersex and mixed-sex individuals in the EE2 group. Among males exposed to BPA, marked gonadal degeneration and signs of disrupted or delayed gonadal differentiation were found. Although 2D:4D did not differ significantly between sexes, both the highest concentration of BPA and EE2 influenced 2D:4D depending on body size, where larger individuals exhibited a lower ratio. These findings suggest that digit ratio dimorphism in amphibians may develop through mechanisms distinct from those in other vertebrates, emphasizing the taxon-specific nature of 2D:4D variation. Overall, the results demonstrate that BPA and EE2 significantly impair gonadal development and 2D:4D digit ratio in a non-model amphibian species, underscoring their relevance for biological risk assessments.

ECOLOGY

Microhabitat partitioning of *Pristimantis*-species across land use types in the Ecuadorian Chocó

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Abstract

Microhabitat partitioning is a key mechanism facilitating species coexistence. While different habitat preferences reduce inter-specific competition in intact ecosystems, they also lead to different levels of vulnerability when facing anthropogenic land-use change. The Chocó rainforest of western Ecuador, a major hotspot of amphibian diversity, is heavily affected by deforestation. Many frog species within this biome belong to the direct-developing genus *Pristimantis*, with often over a dozen congeneric species occurring in sympatry. So far, the mechanisms enabling their coexistence are poorly understood. We assessed microhabitat partitioning of a *Pristimantis* assemblage in two areas within the lowland Chocó, comprising a land-use gradient from pastures, over plantations, to secondary and old-growth forest. While we recorded 13 *Pristimantis* species in old-growth and old secondary forests, only two species inhabited active cacao plantations and only one occurred in active pastures, emphasizing the importance of closed forests for *Pristimantis*. One 'species' occurred along the entire regeneration gradient, however, with two distinct call phenotypes coinciding with different habitat preferences, implying the presence of a cryptic species. In contrast to our expectation, species often overlapped in microhabitat use. Main species-specific differences were in preferred vegetation heights. According to the level of arboreality, our *Pristimantis* assemblages could be assigned to four categories: low, medium and high; as well as species utilizing the full range throughout their life cycle, potentially including an ontogenetic shift. Overall, the level of species-specific differentiation was unexpectedly low and leaves open questions about how the diversity of this genus has evolved and is locally maintained.

MORPHOLOGY

A comparative study on morphological plasticity of *Hynobius* salamanders

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Abstract

Phenotypic plasticity, the ability of an organism to alter traits in response to environmental variation, is well-documented across various taxa and is considered a crucial mechanism for survival in the face of environmental changes. It has been a major topic in evolutionary biology, with many studies examining how plasticity contributes to novel traits and the genetic mechanisms behind it. However, comparative phylogenetic analyses of phenotypic plasticity involving multiple species remain limited, and the macroevolutionary processes related to the acquisition or loss of such plasticity itself remain unclear for many taxonomic groups.

This study focused on the salamander genus *Hynobius*, which has diversified widely in Japan and includes species with larvae adapted to either lentic (still water) or lotic (flowing water) habitats. Among lentic species, two are known to show plasticity: larvae raised under high-density conditions develop enlarged gapes, an adaptation linked to cannibalism. We aimed to investigate how this plasticity evolved across the *Hynobius* phylogeny through experiments on species representing major lineages.

We collected egg clutches of *Hynobius* species from various regions of Japan and reared the hatched larvae under two conditions: single rearing and high-density rearing (with 13 delayed-hatching siblings) from day 10 after hatching. Body length and gape width were measured weekly for four weeks.

In most lentic species, larvae reared at high density developed significantly larger gape widths relative to body length than those reared alone. In contrast, lotic species showed no significant differences in relative gape width at any time point. These results suggest that the plasticity to develop enlarged gapes under high-density conditions is retained in many *Hynobius* lineages. Its absence in lotic species implies that this trait may have been secondarily lost or evolved in parallel among lentic lineages.

CONSERVATION

Role of trade and use in amphibian conservation

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Abstract

A multitude of factors contribute to the decline of amphibians worldwide in such a way that amphibians are one of the most threatened groups of vertebrates today. Therefore, amphibians are subject to many conservation actions and laws in Europe to protect and restore their populations and habitats. In contrast to universal threats, such as habitat loss, the use of and trade in amphibians acts mostly on a local scale, thus population specific as risk factor. But at the same time, it acts in concert with other risk factors for many species. Focussing on Europe, an overview over the various forms and dimensions of the use and international trade of amphibians is provided as well as existing legislations to regulate trade to improve species conservation. Moreover, challenges in protecting amphibians from overharvesting are discussed, along with how different solutions can contribute to integrative concepts for the benefit of amphibian populations in the wild.

ECOLOGY

A plea to unify reporting of body condition values across populations, with examples from *Bombina variegata* and *Bufo viridis*

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Abstract

The Scaled Mass Index (SMI) has become increasingly popular for assessing body condition, especially for amphibians. The SMI allows direct comparisons of body condition values among different investigations of a species, but only if the same scaling exponent and reference length are used in the calculations. The scaling exponent is calculated as the slope of a standardized major axis regression of logarithmic body mass on body size. Using samples that include only a subset of the full body size range (i.e. only juveniles or only adults) may yield deviant estimates for the scaling exponent, not correctly representing ontogenetic allometry. Reference length is an arbitrary value, but using the mean body size is a common practice. Aiming to unify the reporting of body condition indices, we propose reference values (scaling exponent and reference body length) for *Bombina variegata* and *Bufo viridis*, based on data from numerous populations. We demonstrate the pitfalls of applying deviant estimates of scaling exponents when using a reduced range of individual body sizes by reanalysing published results of a common-garden experiment with *B. variegata* metamorphs. We present data on interannual variation of body condition in *B. viridis*, both to caution against rash conclusions on population status from single samples, and to stress the importance of temporal dynamics. To illustrate strengths and limitations of analyzing body condition, we discuss data on the relations of body mass and SMI measured shortly after metamorphosis with survival

in a cohort of free-living *B. variegata*. Using a unified scaling exponent and reference length for each species may allow for meaningful comparison and interpretation of the differences in body condition among populations due to habitat and environmental changes, which is a major concern for the future.

ECOLOGY

Water for LIFE: Restoring Amphibian Habitats on Radensko Polje in LIFE AMPHICON project

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Abstract

Radensko polje, a shallow karst depression in central Slovenia, supports a diverse assemblage of amphibians adapted to dynamic wetland habitats. However, their reproductive success is increasingly limited by hydroperiod instability, particularly in spring, when water levels often drop before larvae complete metamorphosis. Natural breeding sites, locally known as retja or estavelles, are temporary water bodies formed in depressions with variable groundwater input. These sites often dry out rapidly due to climate variability and altered hydrology, leading to frequent reproductive failures. In 2020, early spring desiccation led to widespread reproductive failure across most natural habitats.

Anthropogenic modifications, particularly extensive land drainage and watercourse regulation in the 1960s, have intensified flood dynamics and reduced water retention capacity across Radensko polje. Water now recedes more rapidly, particularly during the critical breeding season, further threatening amphibian populations. Species most affected include *Triturus carnifex* (Italian crested newt) which require sufficiently long hydroperiods for larval development.

As part of the LIFE AMPHICON project, 40 artificial ponds were constructed in strategic locations across Radensko polje to extend hydroperiods and supplement the existing natural network. These ponds vary in depth and substrate characteristics and are designed to mimic natural estavelles while providing more stable aquatic conditions. Monitoring shows that these artificial water bodies are being successfully colonized by multiple amphibian species.

Ongoing research focuses on hydroperiod dynamics, larval development rates, and population connectivity to assess long-term viability of amphibian populations under changing climate and land-use conditions.

ETHOLOGY

Coercive mating in Hermann's tortoise (*Testudo hermanni*) on Golem Grad island affects personality traits of harassed females

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Abstract

The continuum between shy (risk-averse) and bold (risk-taking) personality is one of the most researched personality traits in behavioural ecology. Intrapopulation variation in boldness may be related to various behavioural and fitness-related consequences, such as social interactions, reproductive success and survival. We investigated intrapopulation variability in Hermann's tortoises ($N=12\text{♀}$; 13♂) in relation to the latency for head emergence (HE) when overturned, a measure commonly used to explore the shy-bold spectrum in chelonians. Our results indicate that HE shows high repeatability, with 76% of its variation attributed to differences between individuals and 34% to variation within individuals ($SE=0.07$, $p=0.01$). In addition, HE was significantly shorter in females than in males (mean \pm SD: 42 ± 39 vs. 155 ± 38 respectively; two-way repeated measures ANOVA: $F=4.3$, $p=0.05$). While Golem Grad males exhibit a range of personalities from shy to bold, females are mostly bold. We hypothesise that the phenotypic plasticity of females with respect to boldness is canalised under persistent coercive mating. The coercive mating in this population results in physical wounds in the cloacal region of females, a lower body condition index, lower fecundity and ultimately a higher mortality rate compared to females from a nearby population. It appears that bold females, which immediately emerge and escape when threatened, are less prone to prolonged coercive mating than shy females, which currently appear to be less common in the population.

ECOLOGY

Design and analysis of eDNA surveys for great crested newts

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Abstract

Environmental DNA is becoming a widely used tool to assess the presence-absence and distribution of aquatic taxa. In England, a standardized qPCR eDNA sampling protocol for great crested newts has been licensed for commercial surveys and is now widely used in national and regional surveys. Although this eDNA protocol can be a convenient, cost-effective and highly sensitive method for detecting the presence of great crested newts, as with more traditional survey methods it can suffer from imperfect detection at both the pond sampling and laboratory analysis stages. Such imperfect detection may have consequences for interpretation of the results and subsequent commercial decision making. Here we describe a method for estimating both false positive and false negative rates in a sampling programme and use this to design an optimum eDNA sampling protocol that may minimize the risk of imperfect detection. We also demonstrate how eDNA concentration varies seasonally in relation to adult population size and larval production. Although eDNA can be detected outside the recommended survey window of mid-April-June, we recommend against extending the sampling season because of the higher risk of false negatives at these times. Lastly, we discuss how metabarcoding surveys can provide additional information about aquatic systems and recommend some modifications to the currently used eDNA protocol for great crested newts in England.

CONSERVATION

Innovative Use of Detection Dogs and Camera Traps Reveals Terrestrial Activity and Shelter Preference of Native Newts in a Central European Floodplain

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Abstract

Compared to the well-studied aquatic life stage of amphibians, their terrestrial behaviour remains poorly understood. This gap limits the development of targeted conservation measures and the identification of suitable terrestrial habitats. In 2024, we investigated surface activity of two native newt species (*Lissotriton vulgaris* and *Triturus cristatus*) in the Leipzig floodplain system using two specially trained detection dogs and camera traps. Cameras at four confirmed shelter sites recorded images every minute throughout summer and autumn. Microclimate data, shelter structures, and habitat features were also collected. Results showed that both newt species were subterranean throughout summer. We identified species-specific, temperature-dependent activity thresholds and preferred thermal ranges for surface activity. Precipitation had no significant effect on activity, likely due to the high water-holding capacity of the floodplain forest. Shelter preferences also differed: smooth newts were most often found near root systems, while great crested newts favoured deadwood and fallen logs. Smooth newts additionally preferred areas with lower canopy cover. These insights offer a basis for improved terrestrial monitoring and habitat protection strategies, but should be validated in other habitat types for broader applicability. The novel combination of detection dogs and camera traps offers an effective tool for terrestrial amphibian research and should be considered in future cross-habitat studies.

OTHER

Remote recognition of lizards using UAS

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Abstract

Unmanned Aircraft Systems (UAS) such as drones have proven to be an efficient tool in various research fields of wildlife ecology, such as the monitoring of populations of large mammals. Detecting small ectothermic reptiles however remains a challenge. Without thermal imaging technology a greater number of images obtained at lower flight altitudes is required. This increases the overall effort in both detection flights and image analyses, leading to a low-cost efficiency. As part of a dissertation, it was evaluated to what extent UAS use could replace or complement classic conventional line transect sampling of lacertids i.e., of wall (*Podarcis muralis*) and sand lizards (*Lacerta agilis*). To validate the applicability of UAS for lizard detection flight initiation distance of 40 individuals per species was evaluated during the approach of a light-weight drone (557 g). The maximum flight altitude was estimated at which the animals could still be detected on images taken by the drone camera (8.5 MP resolution). All lizards exhibited a flight response only when the drone approached to a distance of 150 cm or less. In contrast, the visibility threshold for adult lizards measuring around 38 pixels was already reached at a flight altitude of 4 m. Even a 2 m altitude required to detect juvenile individuals remains above the flight initiation distance triggered by the drone used herein. Thus, the detection of wall lizards and sand lizards using drone-based methods appears technically feasible and could be a valuable tool in areas that are otherwise inaccessible for conventional line transect sampling. However, to match or exceed the efficiency of the latter traditional method, the use of larger drones equipped with higher-resolution cameras is required. If these could probably trigger flight responses in larger distances remains to be tested.

TAXONOMY

Taxonomy, Morphology and distribution of the House Snakes of Northeast Africa

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Abstract

Recently, two new species of the genus *Boaedon* were described by the author from Ethiopia and Somalia, which could be differentiated from other congeners by morphology and a high genetic distance. A third new species, called “the Ethiopian black form” is in process of description. Within the genus *Boaedon* we conducted a molecular study based on 126 mitochondrial 16S rRNA sequences, which revealed 23 monophyletic species-level groups in Africa, six of these in East and Northeast Africa. The second new species from Ethiopia is clearly differentiated by genetic distances and morphology from its sympatric congener *B. broadleyi* sp.n. and *B. subniger* sp.n from Somalia.. In addition to the taxonomy, we present the skull morphology of all three new species using μ CT scanning with 3D volume reconstruction and segmentation of the bones.

The morphological variation of the skulls are slightly, but some differences could be found in shape of some bones and different tooth numbers. Moreover an updated distribution map for all *Boaedon* species occurring in Northeast and East Africa is presented.

CONSERVATION

New toolkit, combining photo-ID and camera trapping, boosts efficiency in monitoring of Hungarian Meadow Viper reintroductions

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Abstract

The Hungarian meadow viper (*Vipera ursinii rakosiensis*) conservation program started captive breeding of this unique species in 2004, aiming to save this unique snake from extinction by systematic reintroductions. Over the past two decades more than 5400 vipers were born in the Hungarian Meadow Viper Conservation Centre. Vipers can be individually identified by their unique head scalation, enabling the use of photographic identification, giving low-cost alternatives to marking or tagging. A self-developed MySQL database, Vipera 1.8.3, is enabling the project team to catalogue the vipers, their photos, scale reads and any relevant measurements and events. Reintroduction of the species to reconstructed grasslands was started in 2010 and over 1200 vipers were released to 15 habitats in Hungary in the past 15 years. During regular monitoring, visual encounter surveys and burrow checks by pipe camera, we had 679 viper observations, out of which 447 times we could identify the exact individual. At least once we encountered 15.6% of the released vipers, beside 260 newly registered individuals. In 2023 and 2024 we installed short drift-fences combined with camera-traps in 3 locations, influenced by the Adapted-Hunt Drift Fence Technique (AHDriFT). These camera traps recorded 39 vipers in 2023 and 37 in 2024, out of which we managed to identify the vipers 28 times and 22 times, adding observation records of 20 individuals in 2023 and 12 in 2024, increasing the local recapture rate to 31.0%. With the application of a ruler, we were even able to measure total length and estimate tail length and SVL on the captured images, gaining information on growth rate of identified individuals. In 2025 we installed similar systems to all release sites, providing not only valuable data on the vipers fate, but

other inhabitants of the locations, helping in the estimation of prey and predator presence or abundance.

ECOLOGY

Complexity in environmental variation shapes amphibian metacommunity structure at ponds along a regional urban-rural gradient

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Abstract

Urbanisation modifies and fragments natural ecosystems, shaping local species assemblages that often have non-linear responses to multiple variables. There can be divergent responses within metacommunities along the urban-rural gradient and pond-permanence gradient. However, few studies have explored non-linear and interactive relationships between amphibians and biotic/abiotic factors. Here, we examined relationships between amphibian species richness and occupancy and environmental covariates at ponds in Hungary.

We conducted three surveys for amphibians at 100 ponds around Budapest during the breeding season in 2023. Relationships between the probability of occupancy and covariates were assessed in four multi-species occupancy models, while accounting for imperfect detection. We used estimates of number of species at each pond derived from the models in four respective generalised linear models to examine the influence of each covariate on species richness.

We detected nine species. Mean occupancy and species richness showed negative linear relationships with urban land cover within a 1000-m radius, while both peaked in intermediate-sized ponds. We found a positive linear relationship between distance to the nearest pond and both occupancy and species richness. There was a strong positive relationship for both response variables and water level fluctuation at a pond. Species richness was negatively associated with fish presence and positively associated with aquatic vegetation cover. There was an intermediate response to chlorophyll-a concentrations at ponds. Divergent patterns in occupancy were evident among individual species, particularly with landscape composition and fish presence. Our results highlight the species-sorting perspective of metacommunity theory. We hypothesise that the predicted decrease in occupancy and species richness at closely-spaced ponds was likely due to turnover occurring within dense pond clusters, as well as site fidelity.

CONSERVATION

Targeted connectivity: using selective passages to protect amphibians and halt crayfish spread**Jelger Herder**

RAVON

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Abstract

Invasive species pose an increasing threat to native amphibian populations. In the Netherlands, a rapidly expanding population of marbled crayfish (*Procambarus virginalis*), a parthenogenetic invasive species, was discovered in the Overasseltse and Hatertse Vennen near Nijmegen. This wetland area hosts a unique amphibian community, with eleven species including the endangered common spadefoot (*Pelobates fuscus*) and great crested newt (*Triturus cristatus*). The introduced crayfish present a direct threat to these amphibian populations.

Initial detections of crayfish occurred only in the central part of the area, while the most important amphibian breeding sites are located in the southern part. To prevent further spread of crayfish toward these sensitive locations, a 1.3 kilometer long, 50 cm high physical barrier was installed in 2022.

To maintain habitat connectivity for native amphibians, we developed and tested selective passages: small ramps made of stainless steel, integrated into the barrier, that amphibians can use, but which crayfish cannot climb. Experimental trials showed that crayfish were unable to ascend ramps steeper than 30°, while all tested amphibian species successfully used them. In 2023 and 2024, we tested 20 of these selective passages during peak migration periods. Our results indicate that these selective barriers can successfully limit the spread of invasive crayfish while maintaining essential connectivity for amphibian populations.

In this presentation, we also share practical insights gained during implementation: how to respond effectively upon detecting crayfish, the importance of early detection and action, and how we use targeted eDNA monitoring to improve crayfish detection and guide our management strategy.

MORPHOLOGY

Ecological Constraints and Morphological Diversity in Snakes: Convergence, Divergence, and the Shape of Adaptation

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Convergent evolution, where distantly related species evolve similar traits in response to shared ecological pressures, is a hallmark of adaptive evolution. Snakes, with their conserved body plan but broad ecological diversity, provide a unique model to examine how habitat use and diet shape morphological variation. In two large-scale morphometric analyses encompassing over 400 species (~10% of global snake diversity), we investigated the influence of ecology on head, body, and tail shape. Our results reveal that morphology is only weakly structured by phylogeny and allometry, and is instead primarily driven by ecological specialization. Head and body shapes reflect distinct ecological adaptations: fossorial species exhibit compact heads and bodies suited for burrowing; aquatic snakes possess streamlined forms for hydrodynamic efficiency; and arboreal species show elongated heads and slender necks for navigating complex three-dimensional habitats. Terrestrial snakes, in contrast, display the highest morphological disparity, suggesting greater versatility in response to generalist ecologies. While head shape covaries with diet, species sharing similar diets do not necessarily converge morphologically. Furthermore, convergence is most evident among arboreal and terrestrial species, whereas fossorial and aquatic groups exhibit greater morphological divergence, suggesting multiple functional solutions to similar ecological challenges. These findings underscore the complex interplay between ecology and morphology in snakes, highlighting the dominant role of habitat use over phylogenetic history and the limits of morphological convergence in the face of functional constraints.

CONSERVATION

Slithering on the edge: a scientific chronicle of *Vipera ursinii ursinii* in a warming Central Apennines landscape

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Abstract

In the last years, theoretical and practical conservation efforts for the endangered meadow viper *Vipera ursinii ursinii* in the Central Apennines have shifted from range-wide assessments to fine-tuned spatial strategies. Since 2013, field data gathering started in the Central Apennines, leading to the 2020 review about the conservation status at the European scale, which highlighted threats, knowledge gaps, and urgent needs for this high-altitude specialist. In 2021, niche divergence among Italian and French populations was quantified, highlighting both shared and distinct habitat preferences. Then, a specific post-modelling framework integrating several environmental parameters was published to support fine-scale conservation prioritization. The monitoring and field-data sampling continued, leading to two new studies that further tightened the conservation focus.

The first explores the trophic ecology of *V. u. ursinii* through faecal analyses conducted from 2021 to 2024, which reveal a persistent dependency on certain Orthoptera species, many of which are elevation-restricted and highly sensitive to changes in vegetation. Then, we modelled over 50 prey species and synthesized them into a weighted "typical Orthoptera" layer to refine vipers' SDMs, which we further integrated with NDVI trends. This coupled trophic-climatic approach highlights both persistent strongholds and areas predicted to experience negative change.

The second study quantifies population densities across historical and recent surveys. Comparing sampling results over a 20-year timespan (2005-2025), starting from the earliest ones of the 1920s, we show that many areas exhibit no current occupancy, and densities vary according to the Apennine massif considered. All these integrated approaches highlight ongoing and future declines, underscoring the need for urgent conservation actions.

CONSERVATION

Effects of antifungal treatment and husbandry practices on the skin microbiota the Ornate Forest toad *Rhinella ornata* (Anura, Bufonidae)

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Abstract

Amphibians held under human care are essential for ex situ conservation programs, research, and public engagement. However, maintaining healthy ex situ populations poses challenges, including controlling chytridiomycosis, a disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). The antifungal itraconazole is widely used to treat Bd infections, but its potential impacts on the amphibian skin microbiota remain poorly understood. Standard treatment protocols, which often involve substrate removal, may also disturb microbial communities. We experimentally evaluated the impacts of itraconazole treatment on the *Rhinella ornata*'s skin microbiota through three sequential phases, each lasting 10 days: (1) substrate deprivation, (2) substrate deprivation and daily sterile water immersion, and (3) substrate deprivation and itraconazole exposure. After each phase, animals were returned to standard housing for 40 to 60 days for microbiota recovery. Then, a resilience phase was assessed 60 days after the last of the three phases. Skin swabs were collected before and after each phase and analyzed via 16S rRNA sequencing. Alpha diversity significantly declined following all experimental phases. Beta diversity (Unweighted UniFrac on rarefied data) indicated pronounced shifts in community structure, particularly after itraconazole exposure. Differential abundance analysis (DESeq2) revealed that husbandry interventions resulted in more pronounced changes in ASVs than antifungal exposure. Microbial communities did not return to baseline diversity levels during the resilience phase. Our findings demonstrated that the entire treatment protocol, rather than the antifungal agent alone, can alter the amphibian skin microbiome. Hence, careful planning of antifungal interventions and strategies to promote microbial recovery are essential to minimize unintended impacts on host-associated microbial communities and enhance the effectiveness of ex situ conservation programs.

CONSERVATION

Photographic identification of sand lizards (*Lacerta agilis*) by facial scale markings

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Abstract

Recognition of individual animals is a presumption for capture-mark-recapture studies. Non-invasive techniques like photographic recognition based on naturally occurring markings offer a way to record animals without the stress of capture. In our study, we investigated if photographic individual recognition based on facial patterns in the sand lizard (*Lacerta agilis*) can be conducted successfully in the field. For this, we tested (i) if animals can be distinguished from one another reliably by using the program "I3S Pattern" and (ii) whether it is possible to collect suitable image material for this purpose in the field. We caught 41 adult sand lizards and photographed them in a controlled situation, with images taken from different angles and degrees of coverage from a distance, as well as profile images of captured animals. In a second step we took photos in the field on a study site on three consecutive days to test if adequate image material could be collected for a capture mark recapture study.

Animals were correctly identified by I3S when using photos from the controlled situation, with 95% of the pictures being assigned to the correct animal within the first 5 suggestions. The image material collected in the field revealed good quality for analysis in I3S in 74.4% of cases. Although recognition values by I3S were lower than those in controlled conditions with 83.3% of pictures being assigned to the first five suggestions, all recaptures were recognized correctly. We conclude that photographic identification by facial scale markings is a quick and reliable method for capture-mark-recapture studies on sand lizards.

CONSERVATION

Redesigning artificial nests for the benefit of Japanese Giant Salamander (*Andrias japonicus*) conservation

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Abstract

Japanese Giant Salamanders (JGS) are one of the world's longest-lived and largest amphibians. While they are protected by government legislation, they remain a species in dire need of further conservation efforts due to agricultural runoff and urban pollution, habitat fragmentation, and the threat from hybridization with invasive Chinese giant salamanders. With river infrastructure fragmenting JGS habitats, there are fewer natural nesting sites available and artificial nests are one possible remedy. Such nests have been created in the past but have largely remained unused by JGS. In central Japan's Mie Prefecture, a set of artificial nests was noted to be entirely unused for years, and a functional redesign is of the essence. We analyzed the existing design and consulted experts to understand why JGSs did not utilize these nests, even when no other nesting sites were available. We used our observations, interviews, and literature in a multi-criteria analysis to identify an artificial nest design that optimized materials, reduced environmental impact, accounted for temperature preferences, and any other pertinent parameters. The final artificial nest redesign consists of a structure with one entrance made of gabions, includes a hydronic radiant system beneath the nesting area to maintain a temperature preferred by nesting JGSs, and will be constructed at existing artificial nest sites to avoid broader environmental disturbances.

EVOLUTION

Integrative genomics and morphometrics reveal ancient lineage divergence and morphological stasis: diversification dynamics of the *Pristurus rupestris* species complex in an arid mountain system

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Abstract

Cryptic species challenge the study of diversification by concealing deep evolutionary divergence beneath striking morphological similarity. Extreme environments, such as arid mountain ranges, have been shown to harbour particularly high levels of hidden species diversity. In the Hajar Mountains of southeastern Arabia, the *Pristurus rupestris* species complex harbours multiple deeply divergent lineages exhibiting considerable phenotypic variation among species. Here, we investigate the evolutionary dynamics of this radiation by integrating geometric morphometric analyses with whole-genome sequencing and phylogenomic reconstructions. We analyzed five species – *P. ali*, *P. assareen*, *P. feulneri*, *P. omanensis*, and *P. rupestris* sensu stricto – including all previously identified genetic lineages within those species, and generated the first reference genome for an Old World sphaerodactylid gecko (*P. rupestris* sensu stricto). Morphometric analyses of comprehensive two- and three-dimensional morphological datasets indicate strong overlap in both body and head shape. Despite considerable phenotypic similarities, our results reveal deep interspecific divergences exceeding 16 million years and contrasting demographic

histories. While we detected introgression restricted to specific lineage pairs, phenotypic crypticity persists across the entire complex, suggesting a strong role of stabilizing selection in harsh environments. Our study highlights the *P. rupestris* species complex as an ideal model for investigating the role of environmental constraints in limiting morphological divergence and shaping diversification dynamics in arid mountain ecosystems.

OTHER

Automated Monitoring of Anuran Species Through an Easily Customizable Machine Learning Approach and an Open-source Pipeline

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Abstract

Machine learning (ML), a subset of artificial intelligence, is gaining prominence in the analysis of animal vocalizations. However, many existing ML algorithms are proprietary, require extensive datasets for training, or are not tailored to specific research needs. Additionally, concerns surrounding data sharing and privacy persist. Concurrently, advancements in electronics have enabled cost-effective recording devices collecting extensive audio data, which must then be analyzed—either manually or computationally. As a proof of concept, we developed an ML-based call detection algorithm to facilitate the analysis of field recordings of the European green toad (*Bufo viridis*) during its breeding season. We implemented the algorithm in R, an open-source software platform, and provided detailed instructions for adapting the approach to other animal vocalizations and research questions. Using a dataset of 34 green toad calls and 166 audio files without green toad calls (split into 70% training and 30% testing subsets), the ML achieved 0.99 accuracy. The final algorithm was applied to amphibian recordings newly collected by citizen scientists. The classification function employed three categories: 'Target detected', 'Target not detected', and 'Double check'. Among files containing green toad calls, 90% were correctly classified as 'Target detected', while the remaining 10% were flagged as 'Double check'. For files without green toad calls, 89% were classified as 'Target not detected', with 11% flagged as 'Double check'. Importantly, no files were misclassified. Building on this success, we are currently developing a similar ML algorithm for the common spadefoot (*Pelobates fuscus*) and plan to extend the approach to eight amphibian species found in Austria and Bulgaria. Our findings demonstrate that researchers can develop efficient and accurate pattern recognition algorithms tailored to their specific needs, using accessible tools and open-source platforms.

CONSERVATION

Herpetological Action Plans in Czechia

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Abstract

Most of herpetofauna is strictly protected by law in Czechia. However it itself is not enough in many cases because specific active measures are needed. Thus, we adopted Action Plans scheme for several species (not only for herpetofauna). We have one for *Zamenis longissimus*, which is already ongoing for 14 years. And another one for *Epidalea calamita* is approved by ministry. We also cooperate with our Hungarian colleagues working on *Triturus dobrogicus* for who we supported data about species distribution in Czechia.

Zamenis longissimus is rare in Czechia because there were only 3 populations. However, our AP is working very well. We save the most vulnerable and isolated population near Carlsbad, which was main reason why this AP started. But another populations are subject of this AP too, because main problems are same everywhere: overgrowing of vegetation and lack of small-scale human agricultural activities. So we are building breeding and wintering places, cutting unwanted vegetation, doing research and also working with public because of snakes unpopularity. We do regular monitoring which show us good results. And even in last decade two new population were found what give us new oportunity for improvement and extension of this AP.

Epidalea calamita is different example. Once it was much more widely distributed in north, west and south Bohemia but it disappeared from many localities. Reasons are mainly succession and overgrowing of open space areas related with closing of brown coal and sand mines and their recultivations. There are several local populations yet but their status is bad in their majority. So the main goal of this AP is to preserve as much of them as possible. Activities in first years of realisation will aim on detailed monitoring and evaluation of population's size. Active management is needed to slow down succesion and changes of old recultivation plans too. However, we have now a lot of experience so we hope it will be succesfull too.

BIOGEOGRAPHY

Sticky Innovation, Limited Expansion: Biogeography and Evolutionary Ecology of Toepads in Geckos

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Abstract

Geckos are renowned for their adhesive toepads, which are discussed as a driver of gecko diversification. But the potential correlation of toe morphology with aspects of both the ecological niches in terms of macro- and microhabitat use remained underexplored. Here, we examine the climatic and geographical space occupied by geckos with different toepad configurations and the habitat types they use in order to test whether gecko diversification and niche occupation can be explained by toepad morphology. We coded toepad morphology for all extant species and explored trait evolution, the occupied climate niches and habitat associations using phylogenetic modelling, range maps, climate PCA and hypervolume analyses.

We confirm multiple independent origins of adhesive toepads from padless ancestors but reveal a novel evolutionary transition from distal to proximal toepad configurations. Geographically, species with basal/proximal pads dominate Central African rainforests, species with distal pads are common in Australia and the Caribbean, and species with fanlike pads are restricted to northern Africa and Madagascar. Pad morphotypes are correlated with structurally complex habitats such as savannas, rocky areas, and human-modified environments.

Overall, we find no evidence that adhesive toepads enable access to novel climatic zones. Instead, they appear to evolve within existing environmental regimes, possibly by providing a selective advantage in specific microhabitats. Additionally, the geographic distribution patterns we see today seem to be shaped by biogeographical contingency, with convergent evolution in geographically distinct but ecologically comparable regions. Our findings suggest that adhesive toepads did not support the exploration of new biomes characterized by differing climates, but instead lead to further ecological refinement as the true driver of gecko diversification.

CONSERVATION

Accounting for amphibians' behaviour in drift fence optimisation: an exploratory study

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Abstract

Drift fences are a widely used tool in amphibian roadkill mitigation, aiming at guiding individuals towards traps or tunnels that allow safe passage while preventing access to the roadway. Their effectiveness in preventing roadkill is well established, however, few studies have investigated their guiding function, despite the possibility of informing better designs. To explore this gap, fluorescent paint was applied on the belly and hind limbs of toads ($n=57$), frogs ($n=29$) and newts ($n=17$) captured less than 2 m away from trap-equipped drift fences during the breeding season. The marking left on the ground by the released individuals allowed to indirectly study their movement behaviour. Tracks were segmented and segment length, deviation angles, used and available microhabitats were recorded. Climbing attempts and the last known position were also noted. Segment length and angles were used to calculate a sinuosity index while Chesson's electivity index was used to assess microhabitat selection. Among the 103 recorded tracks, three stereotyped behaviours were identified: migration (70 %), migration withdrawal (7 %), and erratic movement (23 %). The median track length was 8.62m (5.63 – 14.37m). Migration withdrawal generally occurred less than 1m after reaching the fence, while migrating individuals stayed in contact with the fence for 8.94 ± 6.77 m. Bare ground was preferred by migrating amphibians while vegetation was avoided across all behaviours. A GAMM analysis of the probability of capture relative to distance travelled along the fence indicated a 0.63 probability upon encountering a first tarp, and >0.99 when encountering two traps. We therefore advise to place traps every 4-5 m to optimize capture rates. Preventing migration withdrawal seems unfeasible, as it would require a trap approximately every meter.

CONSERVATION

Angolan Geckos: Diversity and Conservation**Javier Lobon-Rovira**

CIBIO

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Abstract

Angola's reptile diversity has historically been underestimated due to limited research, particularly during the civil war (1975–2002). By 2018, only 278 reptile species were documented—far fewer than neighboring countries like South Africa. However, since 2007, renewed scientific exploration and modern molecular techniques have triggered an unprecedented surge in discoveries, especially within the Gekkonidae family.

Post-war efforts have rapidly expanded species records—from just 27 gecko species in 1975 to 59 today. Recent breakthroughs include the description of endemic taxa like *Kolekanos plumicaudus* and *K. spinicaudus*, as well as the rediscovery of the monotypic *Bauerius ansorgii*, a remarkable relic species. Despite this progress, vast regions of Angola remain unexplored, indicating that relevant of its gekkonid diversity is still undocumented.

This study synthesizes the history of Angolan gecko research and assesses their conservation status, emphasizing the country's role as a hotspot of diversification and endemism for pad-bearing lizards. Given the rapid rate of new discoveries, Angola represents a critical frontier in African herpetology, with significant implications for biogeography and conservation.

Keywords: Angola, Gekkonidae, endemism, biodiversity discovery, conservation, biogeography

ECOLOGY

Assessing population dynamics of the European tree frog (*Hyla arborea*) in a protected wetland using PVC shelters and photoidentification

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Abstract

In this study, we investigated (i) the population dynamics of the European tree frog (*Hyla arborea*) and (ii) the effectiveness of artificial shelters—PVC pipes—as a non-invasive monitoring tool in the Škocjanski zatok Nature Reserve (Koper, Slovenia). In May 2022, we installed 40- and 50-mm diameter shelters at 24 sites and regularly inspected them from July 2022 to December 2024. A total of 338 encounters of juvenile and adult *H. arborea* were recorded. Narrower pipes were used significantly more often (1.6-fold), despite no significant difference in body size between frogs occupying the two pipe diameters. We applied capture-mark-recapture analysis using individual photoidentification based on lateral stripe patterns, processed with the Hotspotter software. From 30 successful capture events, 291 photographs yielded 130 individuals (41 males, 39 females, and 50 juveniles, based on first capture). There were 161 recaptures (55.3% overall), with 68 (52.3%) individuals recaptured at least

once; and with a maximum of 8 captures of one male and one female. Juveniles had a lower recapture rate: 39 recaptures (43.8%), with 21 (42.0%) individuals recaptured. Lateral stripe patterns were stable across years, including in juveniles. Of 49 juveniles captured in 2022, 12 (24.5%) were recaptured in 2023 and one (2.0%) in 2024, indicating survival over a span of up to 26 months. All recaptured frogs were found at their original capture sites, except one juvenile that moved once between adjacent sites; showing an extremely high site fidelity that was consistent also between seasons. We observed pronounced year-to-year variation in juvenile encounters: 49 in 2022, none in 2023, and 1 in 2024. This study confirms that artificial shelters are an effective tool for monitoring amphibians in non-breeding habitats, highlights the value of photoidentification for assessing site fidelity, population structure, and long-term dynamics of *H. arborea* across multiple years.

CONSERVATION

No stone left unturned: assessing the role of dry-stone structures on biodiversity

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Abstract

As the single largest land use, agriculture has reshaped landscapes for millennia, contributing to creating distinctive agroecosystems where both biodiversity and cultural services have been favoured. However, trends of intensification and abandonment impact ecosystems and the biodiversity they harbour. Considering biodiversity declines within agroecosystems, keystone structures could be crucial for maintaining high levels of biodiversity. Among these, human-made elements scattered throughout the landscape, such as dry-stone structures, are potential keystone structures. Although dry-stone structures are expected to increase habitat heterogeneity and to play an important role for biodiversity, empirical research has not been adequately compiled and analysed. Here, we conducted a systematic literature review to assess the effect of dry-stone structures, such as dry-stone walls, terraces, wells, and tanks, on biodiversity. We assessed a collection of 7104 papers published between 1960 and 2024 by searching both the ISI Web of Science and Scopus databases. Even though 135 papers fit our definition of dry-stone structures and mentioned their importance, only 35 measured the response of biodiversity. We applied a vote count approach, as well as multinomial and binomial post-hoc tests, to the 35 selected papers. Interest in this topic has significantly increased in recent decades. Our results show that most studies were conducted in Europe, mainly

focusing on plants and birds. While dry-stone structures have a generally positive effect on biodiversity across taxa and types of structure, the studies' narrow focus limits our overall understanding of this relationship. Despite this, our findings provide evidence that dry-stone structures have a strong potential for promoting biodiversity. However, further efforts are required to address the geographical and taxonomical gaps identified and to improve understanding of their effect through more hypothesis-driven research.

ECOLOGY

Unravelling the effects of annual temperature variability on turtle population dynamics.

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Abstract

Understanding the effects of temperature on population dynamics is crucial, particularly for species sensitive to climate variability. In turtles, temperature can influence key demographic variables such as growth, survival, and reproduction through direct and indirect pathways. However, the extent and interaction of these effects remain poorly understood. This study aimed to investigate how annual temperature fluctuations affect growth rates, survival probabilities, and reproductive patterns in a northern population of painted turtles (*Chrysemys picta*). We utilized an integrated state-space modelling approach to analyse a comprehensive 32-year dataset comprising 900 individual turtles. Our approach allowed us to disentangle the pathways through which temperature impacts demographic traits and to quantify the resulting effect sizes. Additionally, we employed an integral projection model to predict future population dynamics under various temperature scenarios. These models enhance our understanding of how climate variability can shape the long-term viability of turtle populations and inform conservation strategies.

CONSERVATION

Utilising Favourable Conservation Status to guide landscape-level species recovery in Southern England

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Abstract

Favourable Conservation Status (FCS) is a fundamental concept shaping conservation policy for habitats and species across Europe, providing a critical benchmark for ensuring long-term ecological resilience. Originating from the Bonn Convention in the late 1970s and later embedded in the European Union's Habitats Directive, FCS has evolved into a guiding principle for both legislative frameworks and practical conservation efforts. Clearly defined conservation goals for a range of different taxa and habitats have become increasingly valuable for guiding proactive conservation measures and providing a framework for measuring changes and initiating specific interventions where necessary. Historically, definitions of FCS have focused on national-level assessments, but there is a growing recognition that landscape-scale perspectives offer new opportunities for effective conservation.

Southern England stands as a compelling case study for the landscape-scale application of FCS, as it hosts the richest herpetofauna communities in the UK. Yet, these valuable communities are increasingly threatened by habitat loss, fragmentation, and human disturbance. This presentation examines how integrating national and landscape-level FCS assessments has informed targeted habitat management, the development of strategic action plans, and the implementation of robust monitoring to guide landscape-level species recovery in Southern England.

Vipers of West Asia Today

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Abstract

Vipers from western-central Asia (Levant, Anatolia, to Caucasus) have experienced a taxonomic high time between the mid-1980s to mid-2000s with the description/elevation of eight new species, and few more added in more recent years. In 2009, the IUCN (Intl. Union Conserv. Nature) published a first Red List with threat assessments for most vipers from that region, compiling the scarce information on biology and distribution. However, the knowledge on species integrity, distribution, and threats portrayed in those assessments is often at odds with biological knowledge (e.g., postglacial species expansion/colonization, carrying capacity, competitive exclusion, species definition/integrity vs. geographic variation, recruitment and dispersal, survival/mortality, etc.). Consequently, the author's team begun to update information on West Asian vipers, conducting > 20 expeditions since 2013 to obtain distribution data, interviewing locals, colleagues, evaluating literature and social media accounts, often contacting respective authors for precise coordinates, and collect some tissue samples for DNA analyses. This all-inclusive method has resulted in > 2000 separate data entries of western Asian vipers with some massive range extensions of 400% to > 4000%. The author will present snippets on rapid data acquisition, and a fresh light about distribution, including 'perplexing' sharp contact zones (e.g., parapatry without environmental demarcation/potential density blocking, etc.), ultimately impacting future threat assessments.

ECOLOGY

Urban oasis? Abundant dice snake (*Natrix tessellata*) populations along artificial lakeside habitats in urban landscapes

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Abstract

Landscape alterations, such as urbanization, introduce novel environmental factors and pose threats to biodiversity. Reptiles, which are ectotherms and sensitive to environmental changes, require a thorough investigation into the factors affecting their populations in urban habitats. The aim of our study was to explore the impact of urbanization at both landscape and local scales on the abundance of an aquatic snake living in a lake characterized by a heavily urbanized shoreline.

To achieve this, we conducted six visual encounter surveys on dice snakes (*Natrix tessellata*) at 25 study sites around Lake Balaton in Hungary. We measured urban land use cover, vegetation cover, road cover, distance from main roads, and city size as landscape-scale covariates. Additionally, we assessed the area of emergent vegetation cover, as well as the area of artificial rock and concrete coastal protection structures, serving as local-scale covariates. Count data from snake surveys were analysed by constructing N-mixture models to estimate abundance and assess relationships between snake abundance and landscape-scale and local-scale covariates.

Our results have revealed a positive correlation between snake abundance and urban land use cover, road cover, proximity to main roads, and the extent of artificial rock and concrete shoreline protection structures. This suggests that artificial environments can serve as suitable habitats, offering new ecological opportunities for dice snakes. Nevertheless, our earlier investigation demonstrated a negative correlation between urban cover, road cover, and the proximity of roads with the fluctuating asymmetry of head scales, indicating there is a detrimental impact on the developmental stability of the species in urban habitats.

Collectively, these findings underscore the intricate nature of urbanization, impacting species at various levels with subtle effects.

CONSERVATION

Bridging ecological research and citizen engagement for amphibian conservation in northern Portugal

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Abstract

Freshwater ecosystems provide essential services and the highest degree of species diversity of any other habitat. They are in sharp deterioration, paralleled by a decline in freshwater species, such as amphibians. Moreover, wetlands and amphibians suffer from a negative public perception that limits the impact of conservation actions. The Mindelo Ornithological Reserve, in northern Portugal, has numerous wetlands, and it is renowned for its herpetological value. Currently, 12 of the 22 species that occur in Portugal can be found in this small protected area. However, drastic habitat alterations are underway, such as the increase of urban pressure, inadequate land management and invasive species expansion. This region represents an excellent model for developing a multidisciplinary approach to bridge the research-implementation gap for long-term effective conservation measures. We are conducting amphibian assessment studies to understand their abundance and distribution in the landscape and how the land-use and land-cover changes might affect the amphibian population. In parallel, we developed a questionnaire to the local communities to understand their knowledge and perception on these species, and we are involving them in the project through science communication activities. We will present the first results on these studies and how we are bridging the gap between ecological research and effective conservation actions. Ultimately, this research project aims to integrate landscape changes analysis and amphibian community characterisation, with the development of citizen engagement activities, through science communication research, to develop science-based measures for freshwater conservation.

EVOLUTION

Phylogeny vs. Selection: What Shapes Sexual Size Dimorphism in Snakes?

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Abstract

Sexual size dimorphism (SSD) in snakes is widely observed yet the intrinsic and extrinsic causes of its evolution are poorly understood. We conducted a broad-scale meta-analysis of SSD across snake species but found no consistent phylogenetic or ecological patterns when analysing data at the level of the entire Serpentes order. To address this, we refined our approach by examining SSD at the family level. Using a phylogenetically informed framework, we assessed phylogenetic signal, models of trait evolution, evolutionary rates, and directional transitions related to SSD. This finer-scale analysis revealed more structured patterns of dimorphism and highlighted key evolutionary dynamics that are masked in broader taxonomic comparisons. From these analyses, we are able to infer whether SSD in each family is more strongly shaped by phylogenetic history or by selective pressures, offering insight into the relative influence of evolutionary constraints versus ecological adaptation. Our findings underscore the importance of taxonomic resolution in macroevolutionary studies and provide new perspectives on the drivers of SSD in snakes.

BIOGEOGRAPHY

Mito-nuclear discordance in a Central African rain forest frog, *Hyperolius kuligae* (Hyperoliidae), uncovers intricate evolutionary pattern

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Abstract

African Reed frogs from the genus *Hyperolius* (Hyperoliidae) represent the most species-rich genus of frogs on the African continent with over 140 described species inhabiting a variety of habitats. Many species are known for their extensive morphological intraspecific variation, including in anurans rare sexual dichromatism, as well as widespread interspecific similarity. One of the species inhabiting the rain forests of Central Africa is the small-sized sexually monochromatic *Hyperolius kuligae*, with presumably disjunct distribution in western (Cameroon, Gabon) and eastern (Uganda) parts of the range. However, recently collected material from the central Democratic Republic of the Congo shows *H. kuligae* to be more widespread than previously thought and suggests its continual distribution across Central African lowland forests. Analysis of a 16S rRNA fragment of mitochondrial DNA distinguished the population occurring south of the wide arc of the Congo River as a divergent lineage. However, subsequent analyses of nuclear DNA challenge these results, showing that the southern population differs only slightly from the population north of the Congo River, from which it probably split during the Pleistocene. On the other hand, available data from GenBank suggest that a previously overlooked diversity dated back to the Pliocene may exist in Cameroon and Gabon, despite relatively uniform mitochondrial DNA. To resolve this evolutionary question in a historical biogeographic and taxonomic context, more samples from other geographic areas need to be examined, and the genetic affiliation of type or topotypic material needs to be identified.

TAXONOMY

Introducing the Secretive Serpents of Paradise, the Elapid Genus *Toxicocalamus*

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Abstract

Toxicocalamus is possibly the largest elapid genus you might never have heard of. It is a genus of rarely seen, vermophagous, oviparous, hydrophiine elapids, endemic to the island of New Guinea and its satellite islands and archipelagos. A century after George Albert Boulenger named the genus in 1896, *Toxicocalamus* contained ten taxa (nine species and one subspecies). However, since 2009 resurgent interest in the genus has elevated this number to 24 species, of which 13 have been examined using molecular data. *Toxicocalamus* is now the largest, non-marine, alethinophidian snake genus in the Australasian-Melanesian region, and it is set to become even larger as further species will be described soon. Yet *Toxicocalamus* also remains a relatively understudied genus.

Toxicocalamus species are unique amongst terrestrial Papuan elapids (except for *Pseudonaja*) in lacking a temporolabial scale between the penultimate and ultimate supralabials. Several members of *Toxicocalamus* are relatively stout-bodied and quite sizeable (1.0–1.2 m TTL), while most species are of slender build and less than 0.5 m TTL and a few are extremely slender “bootlace” species with especially short tails, that may be more fossorial or semi-fossorial in habit. The stouter species exhibit typical colubrid-elapid head scutation; 15-15-15 dorsal scale rows; six supralabials; the cloacal plate consisting of two scales; and paired subcaudals, but other taxa display a great deal of interspecific variation. Dorsal scales are arranged in 13-13-13, 15-15-15, or 17-17-17 rows; subcaudals are paired or single; the cloacal plate is comprised of one or two scales; supralabials number from 4–6, and they exhibit a diverse array of head scute fusions including fused internasal + prefrontal; preocular + prefrontal; internasal+preocular+prefrontal; frontal+supraoculars; supraocular+postocular; and supralabials+temporals, which provide valuable clues that aid in the identification of a specimen in hand.

OTHER

The Reptiles of the Archbold Expeditions to New Guinea 1933–1964: a Summary of the 1930s Expeditions

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Abstract

Most of the natural history collectors visiting the island of New Guinea during the 19th and early 20th centuries focussed on the collection of the four “Bs”: birds, beetles, butterflies and botanicals, although mammals were also collected with vigor. The “lower vertebrates”, reptiles, amphibians, and fish, usually attracted less attention and formed more of a by-catch, with poorly documented metadata (i.e., date and time of collection, collection locality).

One major exception were the Archbold Expeditions, a series of seven expeditions that bookended the Second World War: three expeditions during 1933–1939 and four in 1953–1964. They were financed by the heir to the Standard Oil fortune, Richard Archbold (1907–1976), who personally led the first three expeditions, which made substantial collections for the American Museum of Natural History in New York. Although the participants were still primarily interested in mammals, birds, and botany, they also collected many herpetological, ichthyological, entomological, parasitological, geological, and ethnological specimens, most of which were diligently documented. The combined total for the herpetological collections from all seven expeditions, numbered 9,084 specimens, comprising 6,396 amphibians and 2,688 reptiles. The reptiles included 32 turtles in six genera and three families; nine crocodilians in a single genus and family; 2,092 lizards in 31 genera and five families; and 555 snakes in 28 genera across eight families.

Today the herpetological collections from the Archbold Expeditions provide an extremely valuable resource for the study of the herpetofauna of the largest tropical island on Earth, especially since they include ten holotypes and 222 paratypes.

I summarise the first three expeditions and the 979 reptiles collected during those explorations, of which the latter two were the first tropical expeditions to use flying boats to recce, insert, resupply, and extract.

EVOLUTION

Metabolic and fitness consequences of mitochondrial introgression from *Pelophylax lessonae* into *P. ridibundus*

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Abstract

Although mitochondrial introgression across species boundaries is commonplace in amphibians, its metabolic and fitness consequences are not well-understood. Mitochondrial introgression from *Pelophylax lessonae* (L-mtDNA) into *P. ridibundus* is widespread across northern central Europe, despite substantial mtDNA divergence (12% average across the mtDNA genome) between the species. We tested the hypothesis that mito-nuclear incompatibility in *P. ridibundus* frogs with L-mtDNA will alter metabolic and growth rates of tadpoles and lead to higher mortality over embryonic and larval development. We obtained *P. ridibundus* with introgressed L-mtDNA via crossing experiments that leveraged the hemiclonal inheritance of the hybrid *P. esculentus*. We compared the experimental group with the control consisting of *P. ridibundus* frogs with native R-mtDNA. Introgressed tadpoles exhibited significant, temperature-dependent shifts in routine metabolic rate (MR): at 16°C, tadpoles with L-mtDNA showed lower MR than those with R-mtDNA, while at 24°C and 28°C this pattern was reversed. At 20°C, MR was similar for both mitotypes. Mortality rates, duration of larval phases, and overall survival probability did not differ significantly between mitotypes, although there was a consistent trend towards increased embryonic mortality and reduced larval survival and recruitment at metamorphosis in introgressed individuals, particularly under cooler conditions. Growth trajectories were parallel between mitotypes, but introgressed tadpoles maintained lower overall body mass. Our results show that introgression of L-mtDNA into *P. ridibundus*, occurring in many natural frog populations, affects tadpole

performance and fitness and may have temperature-dependent, eco-evolutionary consequences.

MORPHOLOGY

Fine innervation of the contact surface of adhesive lamellae of Tokay Gecko (*Gekko gekko*)

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Abstract

Gecko adhesion has long fascinated scientists across disciplines. By now, the biomechanics and physical basis of adhesion via hierarchical setal arrays have been well-established. However, little is known about how geckos neurally control adhesive contact, especially in response to the complex microreliefs of natural surfaces.

In this study, we investigated the fine neural architecture associated with the adhesive apparatus of *Gekko gekko*.

The aim of our study is to reveal neural supply of the adhesive apparatus of *Gekko gekko*.

Using immunohistochemical labelling for neural markers in combination with confocal laser scanning microscopy, classical histology, and scanning electron microscopy, we found a complex network of nerve fibers branching beneath the adhesive lamellae. These arborizations terminate in discoidal sensory endings embedded near the base of the setal fields.

Based on comparison with previously described sensory structures in geckos and other reptiles, we propose that the observed discoidal endings belong to a mechanosensory system specialized for adhesive modulation and potentially involved in tactile sensing. Assuming this mechanosensory role, we hypothesize that these endings contribute to the fine-scale regulation of the toe pad adhesive contacts.

Our findings open a new conceptual view of gecko adhesion as a neurally modulated process that may involve active sensory feedback and emphasizes the potential importance of sensory structures in dynamic control of contact mechanics. In its turn, this raises new perspectives for design of bio-inspired adhesives with adaptive or responsive properties.

BIOGEOGRAPHY

Goa - more than a tourist spot: mapping the Reptilian fauna in Goa, India

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Abstract

The present provides the first comprehensive and systematic assessment of the reptilian fauna of Goa, India, documenting 83 species across 21 families and 54 genera. The dataset includes new distributional records—most notably, five snake species reported from the state for the first time—as well as observations of atypical morphological variants and the rectification of several previously misidentified taxa. The survey encompasses representatives from geckos, skinks, agamids, crocodilians, and a broad assemblage of snakes. Furthermore, the study introduces the first habitat-specific reptile species richness analysis for Goa, based on IUCN habitat classification codes. Verified records from citizen science platforms such as iNaturalist and the Reptile Database were integrated to augment the primary data. The resulting annotated checklist offers a critical baseline for future herpetofaunal research, conservation strategies, and biodiversity management within this underexplored biogeographic region.

CONSERVATION

Why road mitigation is fundamentally different for newts

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Abstract

Road impacts are much better understood for amphibians and there are now many implemented examples in Europe of road mitigation designs (primarily fence and tunnel systems) that can effectively prevent road mortality and ensure safe passage of a generally high proportion of individuals. However, important issues remain on insufficient implementation standardisation, flawed or untested mitigation designs and poor maintenance, all which can lead to ineffective solutions. In addition, unclear measures of success and weak monitoring design reduce our understanding of effectiveness as well as in-depth ecological separation between species and species groups. For European newts, there is a scarcity of studies from continental Europe, but the existing evidence from Britain all points towards fundamental differences in how these species use and should be incorporated in road mitigation measures, with contrasting seasonal and ecological patterns of use compared to anuran species at the same sites. Implementing tested ecological knowledge is critical for increasing the effectiveness of road mitigation schemes for amphibians and requires transparent guidance for decision-making by practitioners.

CONSERVATION

Frogs, AI and climate change: novel tools can dramatically improve citizen science**Silviu Petrovan**

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Corresponding author: silviuvet@yahoo.com**Abstract**

The popularity of garden ponds has provided significant habitats for common frogs (*Rana temporaria*) and, in Britain, has helped to offset pond loss in the wider countryside, with citizen science data increasingly deployed to monitor wide population trends in urban areas. However, our understanding of the dynamics of such populations over time remains weak and this is particularly pressing now given the rapid climatic changes, with increasingly hot summers and mild winters which could heavily impact survival and recruitment. Using the results of a long-term and intense (weekly) mark recapture study in an urban setting offers substantial opportunities to test and calibrate technological advancements in Machine Learning (ML) to support integration of deeper demographic data collection as part of citizen science efforts. This dataset of over 1500 tagged recaptures of 145 individuals since 2018 highlights novel information on frog pigmentation pattern change recognition, annual survival and individual recognition using AI. It also demonstrates a clear shift of increasingly early spring breeding and apparent disappearance of hibernation. Linking citizen science with novel ML approaches for pattern recognition could dramatically improve our understanding of species ecology and climate change impacts and potential for local mitigation.

ECOLOGY

Uncovering a warm climate admixture clade at the southern distribution range edge of a cold adapted snake, the adder (*Vipera berus*)

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Abstract

Vipera berus is a wide ranging and well-studied cold climate adapted reptile species. It occupies high altitude and near-Artic regions beyond what any other snake can tolerate in Europe and Central Asia, while in southern parts of the range it is generally restricted to mountains. However, many populations in Europe are now fragmented and declining and this is predicted to worsen due to climate change. In 2019-2025 we used opportunistic and targeted surveys and identified multiple new populations forming a previously unknown adder genetic clade from south-western Romania. Unlike other adder populations in Romania, the novel populations were found predominantly in warm, lowland areas (80-250m asl), mainly in or around farmland, and are phenotypically similar to Balkan adders, *V. berus bosniensis*. Microsatellite and NGS analyses confirmed these to be a novel clade in a large admixture zone between *V.b berus* and *V.b. bosniensis*. We hypothesise that this new lineage represents a post-glacial refugia clade that has become isolated due to its strongly diverging climatic preferences in this region of high genetic diversity for adders. The full distribution extent is unclear but most likely extends into nearby Serbia and possibly Hungary. Beyond the biogeographic interest, this lineage represents an important example of long-term adaptation to substantially warmer climatic conditions compared to other nearby and distant populations. Such population level genetic diversity could gain increasing relevance given the rapid climatic changes currently threatening numerous species with regional extirpation.

CONSERVATION

The island-dwelling harlequin toad (*Atelopus gracilis*): from species redescription and ecology to conservation action

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Abstract

Currently, we are globally witnessing severe declines of biological diversity. Among the vertebrates, amphibians are declining at dramatic scales with 35% of its species in a threat of extinction or already extinct. Neotropical harlequin toads, genus *Atelopus*, stand out as the face of this global declines. Since most of the species are experiencing serious extinction risks mainly due to the threat of the invasive chytrid fungus *Batrachochytrium dendrobatidis* (Bd) and its associated disease. In situ and ex situ conservation measures are badly needed. However, remarkable knowledge gaps on the life history of most of *Atelopus* species still persist that prevent the development of concrete conservation actions.

Here I present the taxonomic redescription of *Atelopus gracilis*, a species long synonymized with *A. elegans*, now recognized as endemic to Gorgona Island, Colombia. This taxonomic clarification has immediate implications for conservation, given the species' restricted range. I combined environmental DNA, skin samples and morphometrics to address disease dynamics. In parallel, I conducted a pilot study on movement patterns focusing on differences between males and females and the potential influence of human disturbance near touristic trails. Preliminary results suggest persistent, low-intensity infections with no visible population decline but potential vulnerability given its sedentary behavior.

The results support the classification of *A. gracilis* as Vulnerable under IUCN criteria and highlight the importance of integrating taxonomy, disease surveillance, and behavioral ecology to develop effective conservation strategies. This work contributes a replicable framework for addressing amphibian conservation challenges in other biodiversity hotspots.

CONSERVATION

Crossing Roads and Restoring Habitats: Slovenia's Amphibian Conservation Journey

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Abstract

The LIFE AMPHICON project (LIFE18 NAT/SI/000711, 2019–2026) improves the conservation status of amphibians in four Natura 2000 sites in Slovenia through pond and habitat restoration, better connectivity, and road mortality reduction.

Amphibian populations in these sites are declining mainly due to habitat loss in agricultural landscapes, worsened by climate change, roads, urbanization, and poor water management. Forests also provide crucial habitats, and forestry can support conservation by preserving and restoring forest ponds and nearby areas.

The project applies a holistic approach, combining 130 pond creation and restoration with large-scale terrestrial habitat improvements on 45 ha. In addition, targeted road mitigation measures were implemented on five roads in Slovenia. A total of 135 amphibian tunnels and 14,360 meters of fencing were installed along four of the most problematic road sections (7,020 meters in total).

Project experience was also transferred to Kozjansko Regional Park, where four tunnels and 940 meters of fencing were installed along a 560-meter road section near the Žabja Domačija (Frog Homestead) info center, serving as a demonstration site.

All actions are based on up-to-date population and habitat data, best practices, and expert supervision. Monitoring ensures evaluation and refinement of conservation measures.

Knowledge transfer, public awareness, and education are key to promoting replication and public engagement. To support these efforts, the Coordination Centre for Amphibians (CCA) has been established as Slovenia's national hub for amphibian conservation. The CCA provides expert support, coordinates citizen science initiatives, and connects key stakeholders — ranging from Natura 2000 site managers and road authorities to NGOs, volunteers, and amphibian supporters.

Through collaboration and outreach, the project fosters greater awareness and conservation of amphibians in Slovenia.

CONSERVATION

Genetic defragmentation in a reintroduction of the imperiled European tree frog

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Abstract

Reintroduction is a conservation measure to re-establish and reconnect populations of endangered species. While not always successful, increasing evidence suggests that population size and genetic diversity are important factors associated with long-term maintenance of reintroduced populations. Here we report on a reintroduced population of the European tree frog in Lower Saxony, Germany. Between 2005 and 2008 more than 8500 tadpoles from five source population have been introduced into a former tree frog habitat in a protected area at Lake Steinhude. Since then the population is growing constantly and population size was approximately 20.000 frogs by 2024. In 2015, a population genetic study with microsatellites verified population structure and genetic diversity in comparison to the source populations. The reintroduced population at Lake Steinhude showed ample admixture of the genetic source populations. Almost all sampled individuals ($N = 197$) were descendants of multiple gene pools. The genetic diversity was as high as or higher compared to the source populations. The capacity of the habitat at Lake Steinhude appears to have been reached as tree frogs are leaving the area and colonizing suitable nearby habitat. Compared to other reintroductions of amphibians the success of our project suggests introducing large number of tadpoles together with genetic defragmentation as encouraging strategy for endangered species.

OTHER

Variable Paths to Fertility: Hybridogenesis and Genomic Disruption in Water Frog Hybrids (*Pelophylax esculentus*)

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Abstract

In *Pelophylax esculentus*, a natural hybrid of *P. lessonae* and *P. ridibundus*, reproduction avoids typical hybrid sterility by eliminating one parental genome and clonally transmitting the other. Successful reproduction depends on the presence of one or both parental species, resulting in variable gametogenic pathways. To explore this variation, we examined meiosis of di- and triploid hybrids from Central and Eastern Europe using fluorescent in situ hybridization (FISH). Because genomic introgressions may disrupt gametogenesis, we also assessed genome integrity via comparative genomic hybridization (CGH) on gonadal and somatic tissues.

FISH revealed that most diploid hybrids in Central European *P. lessonae* - *P. esculentus* populations followed canonical hybridogenesis, eliminating the *lessonae* genome and producing haploid *ridibundus* gametes. In *P. lessonae* - *P. esculentus* - *P. ridibundus* populations (Central Europe) and *P. ridibundus* - *P. esculentus* populations (Eastern Europe), we observed broader variability. Some males produced both *ridibundus* and *lessonae* gametes; others failed to eliminate either genome, forming diploid or aneuploid gametes. In two Eastern populations, bivalent-like associations between diverged chromosomes were observed in both di- and triploids.

CGH identified large-scale chromosomal rearrangements and introgressions, including entire *ridibundus* chromosomes in *lessonae* gametes and segments from closely related species (*P. cf. bedriagae*). These alterations were often linked to failed genome elimination and disrupted meiosis. In several triploids, diploid gametes and abnormal

chromosome sets suggest that misregulated hybridogenesis may contribute to triploid formation.

Our results demonstrate that reproductive strategies of *P. esculentus* are population-specific and shaped by local genomic architecture and hybridization history. Introgressions and rearrangements can compromise hybridogenesis, driving reproductive diversity and instability.

CONSERVATION

High grazing pressure is a threat to declining grassland specialist reptiles

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Abstract

Grazing is a dominant factor forming grassland ecosystems, impacting vegetation dynamics and consequently, the population parameters of grassland specialist animals. This study investigates the effects of grazing pressure on three grassland specialist reptile species across four years (2021-2024): the Meadow viper (*Vipera*

ursinii), Balkan wall lizard (*Podarcis tauricus*), and Sand lizard (*Lacerta agilis*). We used multi-season occupancy models to analyse the effects of grazing pressure, grassland wetness, and naturalness on occupancy, colonization, and extinction probabilities. Increasing grazing pressure negatively affected the occupancy of all three species. Meadow viper occupancy was highest at intermediate wetness levels and positively related to naturalness, while grazing negatively impacted occupancy and increased extinction probability. Balkan wall lizard occupancy was negatively affected by wetness and positively by naturalness, while grazing pressure decreased occupancy and colonization probability. Sand lizard occupancy was positively related to wetness, while grazing negatively affected occupancy and increased extinction probability. This highlights how the impact of grazing on reptile populations depends on the combined influence of habitat characteristics. To balance agricultural needs with reptile conservation, we recommend fine-scale grazing systems and the establishment of grazing exclusion zones within critical habitats. Further research should focus on the long-term effects of grazing, combined with fire regimes and climate change.

CONSERVATION

Extensive restoration of the entire habitat complex is key to the successful recovery of threatened species: the case of the natterjack toad *Epidalea calamita* at the northern range margin

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Abstract

Increasing concerns about biodiversity loss have prompted international efforts to restore degraded habitats. Unfortunately, wildlife habitat restoration often fails due to inadequate planning, inappropriate scale, or lack of providing key elements of habitats. We investigated the effectiveness of habitat restoration of a threatened amphibian species, the natterjack toad *Epidalea calamita*, in Estonia, comparing two different restoration approaches – partial habitat restoration and extensive habitat restoration over 16 years. We investigated the impact of these two different approaches on the populations, to find out which habitat components play a key role in restoration and which measures are of primary importance in the restoration of habitats for small amphibian populations. We used GIS analysis to identify changes in the area of the habitat complex and breeding sites in seven natterjack toad locations following the restoration activities. To assess population response to habitat restoration, we used the census of the natterjack toad egg-strings as a proxy to the population size. The average number of egg-strings increased by 1.2-fold after partial habitat restoration and 3.5-fold when comparing partial habitat restoration with extensive restoration. Partial habitat restoration, which consisted of improving the condition of extant habitat, had no significant effect on population size, while extensive habitat restoration, which included improving and enlarging the entire habitat complex led to population growth. The habitat features that positively influenced population growth were the area of entire habitat complex and the number and size of the breeding sites. In terms of habitat restoration and conservation measures, the number of natterjack toad populations was positively affected by restoration of the entire habitat complex (terrestrial habitats and water bodies), which was supplemented by the supportive rearing of tadpoles.

ETHOLOGY

Paternal care as a source of key skin microbiota in a poison frog

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Abstract

Close interactions between parents and offspring play a key role in the transfer of gut microbiota, but we have limited knowledge about their role as vectors for microbial colonization on skin. We performed a cross-foster experiment using a Neotropical poison frog and identified that parental care in the form of tadpole transport by both natural and foster fathers was a key source for microbial inoculation to tadpoles and that transferred communities were maintained until metamorphosis. Microbial contribution by parents included several bacterial genera previously shown to be effective against the chytrid fungus *Batrachochytrium dendrobatidis* (Bd). By contrast, the microbial community of tadpoles that were manually transferred to the provided water bowl after hatching showed a low presence of protective bacteria. These findings provide novel insights into the multifaceted roles of parental care for equipping offspring with essential resources, such as diverse and targeted skin microbial communities.

MORPHOLOGY

3D and 2D morphometric analyses corroborate inter- and intraspecific divergences in an endemic gecko genus in Seychelles

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Abstract

Accurate species delimitation is essential for effective biodiversity conservation, yet minimal morphological variation between cryptic species often obscures true diversity. In such cases, identifying reliable morphological criteria for species delimitation becomes critical for guiding conservation efforts. In Seychelles, reptile taxonomy remains largely unresolved, partly due to phenotypic crypsis. Despite the growing recognition of genetically distinct lineages, which mainly reflect geographical patterns, formal taxonomic revisions are lacking, thereby delaying conservation assessments

for many range-restricted endemics. The *Ailuronyx* geckos of the Seychelles' granitic islands exemplify this problem. The genus comprises ancient, deeply divergent lineages with cryptic species boundaries and frequent misidentifications, complicating assessments of their distribution and conservation status. To investigate patterns of morphological divergence, we applied 3D and 2D morphometric analyses across the archipelagic range of the three recognised species: *A. seychellensis*, *A. tachyscopaeus* and *A. trachygaster*. 3D geometric morphometric analyses of cranial and mandibular landmark data from 42 geckos showed species-level differences, although the limited sample size likely constrained our ability to detect cryptic species. In contrast, analysis of body size-corrected multivariate 2D morphometric measurements from 567 geckos provides strong support for the hypothesised northern and southern islands divergence within *A. seychellensis* and *A. tachyscopaeus*, as well as evidence for population-level divergence of *A. tachyscopaeus* on the largest island. Our findings highlight distinct population units that warrant further genomic investigation and prioritisation for conservation.

ECOLOGY

Life-history variation in two geographically close populations of the eastern sand lizard, *Lacerta agilis exigua* following an extreme climatic event

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Abstract

Recent decades of rapid climate change have been marked by an increase in both the frequency and severity of extreme climatic events (ECEs), such as the catastrophic floods in Spain in October 2024 and the record-breaking heat wave that affected European Russia in July–August 2010. The consequences of ECEs for wild populations, particularly reptiles and amphibians, remain even less understood than the effects of gradual warming. We assessed potential effects of the 2010 heat wave on the lizard *Lacerta agilis exigua* using data on adult body size (over 420 individuals) and reproductive traits (nearly 150 egg clutches laid by field-caught females and incubated under standardized conditions) collected between 2011 and 2015. Two populations from the Middle Volga region—one occupying a drier and the other a more humid habitat—were studied.

In both populations, reproducing females in 2011 exhibited smaller snout–vent length (SVL) and post-oviposition body mass compared to subsequent years. We hypothesize that this reflects a cohort effect: the 2-year-old females—typically the dominant reproductive age class—could exhibit reduced body size in 2011, since they experienced the 2010 heat wave as yearlings, a life stage marked by rapid growth.

Other between-year patterns were less consistent. Male body size was reduced in 2011 compared to 2012–2013 at the drier but not at the humid site. Conversely, egg and hatchling mass were lower in 2011 only in the humid site. Clutch size and clutch mass, adjusted for maternal size, did not vary markedly among years.

Overall, our study provides solid evidence of a short-term but notable reduction in adult body size following a historic climatic extreme. The observed recovery in subsequent years highlights both the resilience and vulnerability of life-history traits in lizard populations exposed to extreme environmental stress. This study is supported by the German Research Foundation (DFG, grant RO 4168/1-3).

ECOLOGY

Population of origin influences early life-history traits in the Common Toad (*Bufo bufo*)

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Abstract

The biodiversity crisis is one of the great challenges of the 21st century, involving the loss and decline of populations and species. Common and widespread species that are decreasing offer us the opportunity to study drivers and processes behind population declines comprehensively and in depth, across multiple populations. Such drivers can be divided into environmental factors, such as land-use, pollution or infections, and genetic factors, such as genetic drift, inbreeding or selection. These factors then influence vital rates within certain demographic stages of a species, which ultimately affect population trends. The Common Toad is one of these widespread species with both declining and stable populations occurring within Europe. To determine the effect of population of origin on early life-history traits in Common Toad, we sampled 160 egg strings from 16 populations with declining or stable population trends in Flanders (Belgium) and reared them in a common garden setup until metamorphosis. We determined the hatching success and time to metamorphosis, as well as the number of surviving animals and their size once every two weeks. Our results suggest a difference in tadpole mortality between populations, which can be linked to the declining status of the population of origin. These results could potentially be linked to maladaptation to the original environment or inbreeding depression, but possible parental effects cannot be excluded as well. Our study demonstrates that early life-history traits in the Common Toad depend on the population of origin and its trend.

CONSERVATION

Reviewing the ecological and economic impacts of *Trachemys scripta* in Europe in the last 25 years

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Abstract

The introduction of exotic species, alongside habitat degradation and global warming, is one of the most pressing issues in invasive and conservation ecology. *Trachemys scripta*, listed among the 100 Worst Invasive Species, is a freshwater turtle which, after decades of pet trade and releases, is now spread almost worldwide. The species possesses a remarkable ecological plasticity and is also suspected to be one of the causes of the decline of European freshwater turtles (EFT). This assumption led to the implementation of significant eradication efforts across Europe. However, understanding the true impact of *T. scripta* on the invaded environments remains difficult due to the co-occurrence of multiple factors such as habitat loss and pollution. For this reason, we conducted a critical review of scientific literature examining the interaction between *T. scripta* and EFT. We assessed whether the study environment was artificial or natural and whether it concerned direct interaction between *T. scripta* and EFT to evaluate its relevance for the conservation of the latter. Additionally, we assessed the financial costs of control and eradication programs of *T. scripta* within EU-funded LIFE projects.

Among the 39 studies, 10 demonstrated an actual negative impact of *T. scripta* on native species. However, study relevance highly depended on the context. Cost analysis from 13 LIFE projects revealed a total cost of €1,951,670, with an average removal cost of €331 per single turtle. Using an objective synthesis of the ecological and economic impact of *T. scripta* in Europe, we discuss targeted, context-specific, and cost-effective management strategies, based on precautionary action and realistic assessments of impact and feasibility.

ECOLOGY

Wildfire impacts on gut microbiota and parasite dynamics in the lizard *Podarcis lusitanicus*

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Abstract

The increasing frequency and intensity of forest fires are transforming ecosystems, particularly in fire-prone areas like northern Portugal. These disturbances modify habitats and promote early successional vegetation, often leading to declines in species dependent on mature habitats while favoring those adapted to modify environments. The native lizard *Podarcis lusitanicus*, which prefers rocky habitats, may benefit from post-fire landscapes due to the increased availability of suitable shelter.

This study investigates how fire affects both the gut microbiota and parasitic communities of *P. lusitanicus*. In spring 2023, lizards were sampled from 12 sites, including areas burned in 2022 and 2016, as well as adjacent long-unburned zones. Ectoparasites and blood samples were collected and analyzed with microscopy and molecular methods. Cloacal swabs were used to assess bacterial communities through metabarcoding of the V4 region of the 16S rRNA gene. Our results showed that microbial composition was influenced by time since fire, though predicted functional profiles of the microbiome remained relatively stable. Microbiota variation was also shaped by sex, body size, and diet, with females showing higher microbial richness.

In parallel, we aim to evaluate how fire affects parasite presence. Our hypothesis is that parasite loads, particularly ectoparasites like ticks and their associated blood parasites (e.g., *Hepatozoon*), are reduced in recently burned areas due to the disruption of their life cycles, which often include free-living stages.

Together, this work will contribute to a better understanding of how environmental disturbances such as wildfires affect both host-associated microbial communities and

parasite dynamics. These insights are essential for predicting how small vertebrate populations may adapt to changing fire regimes.

CONSERVATION

Assessing the effectiveness of a *Xenopus laevis* eradication program using eDNA monitoring

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Abstract

The African clawed frog (*Xenopus laevis*) is an invasive species in Portugal that has successfully colonized two small stream basins, where it dominates ecosystems to the detriment of native fish and amphibian species. To address this issue, a control and eradication program has been implemented, and the species is currently confined to a few locations. However, there are areas where specimens are not captured

because it can be difficult to detect them using conventional methods (e.g., electrofishing), due to factors such as depth, inaccessibility, and dense vegetation.

To evaluate the success of the program, another practical approach is required to determine, with high confidence, whether the absence of captures at a given site reflects true local eradication or the species' elusive nature. One promising tool for detecting species without extensive fieldwork or trained researchers is environmental DNA (eDNA). This method involves collecting water samples from the environment and testing for the presence of species-specific DNA.

We sampled over 30 locations across the invaded basins and nearby streams, differing in time since the last capture of individuals (an interval between 0 and 11 years). Water was filtered using cellulose nitrate filters with a 45 µm pore size to collect waterborne DNA, which was then analysed via quantitative polymerase chain reaction (qPCR).

Our results indicate that the eradication program has been highly successful, markedly reducing the *X. laevis* invasive range. Furthermore, our findings suggest that true absence can only be confidently assumed after at least five consecutive years with no detections using conventional methods. These insights will help guide future removal efforts by focusing resources on specific target areas, thereby increasing the program's efficiency and bringing the goal of complete eradication of *X. laevis* in Portugal closer to reality.

EVOLUTION

Amphibian-specific structural proteins contribute to the formation of claws and nuptial pads in frogs and salamanders

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Abstract

The cornification of epithelial structures in the form of scales, feathers and hair is prominent in reptiles, birds and mammals. Many protein components of these cornified skin appendages are encoded by gene clusters, suggesting tandem gene duplication as a major evolutionary mechanism either to increase gene expression levels or to facilitate subfunctionalization of genes. Here, we report that an amphibian-specific gene cluster contributes to cornification in anurans and salamanders. Cornified claws, nuptial pads and soft-cornified skin of *Xenopus* frogs were subjected to RNA-sequencing and comparative transcriptome analysis. We detected a previously uncharacterized family of mRNAs that encode proteins with sequence similarities to cornification proteins of amniotes. The corresponding genes are arranged in cluster which we tentatively name amphibian specific epidermal differentiation complex (AEDC). These genes were expressed in the skin but not in other organs. mRNA in situ hybridization revealed that one of the AEDC genes is expressed in the cornified epithelial cells of the claws and nuptial pads. The promoter of this gene contains putative binding sites of the transcription factor Hoxc13. CRISPR-Cas9-mediated inactivation of Hoxc13 led to the downregulation of AEDC gene expression and suppression of claw development. Homologs of AEDC genes are present in the genomes of salamanders and caecilians, and RT-PCRs showed that at least one of these genes is expressed in the claw-like toe tips of the axolotl. We conclude that amphibian-specific proteins contribute to the cornification of epithelia in frogs and salamanders.

ECOLOGY

Learning from Strinati's Cave Salamander long-term research

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Abstract

The value of long-term ecological research in investigating evolutionary change, population dynamics, and the impact of extreme or rare climatic events on biological communities is well recognized. However, long-term studies are scarce in subterranean environments that are widespread but relative neglected. In this study, the dynamics of a population of Strinati's cave salamander *Speleomantes strinatii* (Aellen, 1958) was studied over 30 consecutive years. The study site is the Biospeleological Station "Arturo Issel" (Province of Genova, NW Italy), an air-raid shelter used by civilians during World War II. This underground laboratory is managed by a NGO Speleological Group and is entirely dedicated to the study of the resident population of cave salamanders *Speleomantes strinatii*. Starting from 1996, salamanders were captured during three temporary removal occasions to estimate population size, demographic composition and spatial distribution along the tunnel. The salamander population fluctuations were modelled by means of autoregressive moving average time series analysis. During this study, the tunnel entrance was consolidated with concrete and brick walls to ensure secure access to scientists, students and visitors. Therefore, it was also possible to evaluate the salamander population's response to this habitat modification.

MORPHOLOGY

Homology of the shoulder joint and forelimb muscles in frogs and toads (Lissamphibia: Anura)**Robin Saulnier Masson**

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Abstract

It has recently been revealed that there are inaccuracies in the naming and homologization of shoulder joint muscles within Anura and that muscle ontogeny and innervation are valuable to derive hypotheses on muscle homology. To expand the phylogenetic range of previous work and add the muscles of the forelimb, the ontogenetic development, innervation, and morphology of the shoulder joint and forelimb muscles were described based on tadpoles (from Gosner Stages 31 to 41) and adult specimens of 11 species across the anuran phylogeny. Digital dissections of volumes acquired by histological serial sectioning, episcopic microtomy, and contrast-enhanced micro-computed tomography scanning were performed and three-dimensional reconstructions were generated. The results indicate that, like in all tetrapods, the muscles of the shoulder joint and forelimbs are ontogenetically derived from the dorsal and ventral pre-muscle masses in the developing limb bud, which split up and differentiate into individual muscles during ontogeny. The splitting pattern of the pre-muscle masses during ontogeny appears to be mostly conserved across the anuran phylogeny. However, interspecific variations are observed in the muscles of the shoulder joint, where some homologous muscles are characterized by a subdivision into two distinct units. For example, the *m. supracoracoideus* remains undivided in *Xenopus* ssp., while it differentiates into distinct *portio anterior* and *portio posterior* in *Rana temporaria*. In contrast, interspecific variation among forearm muscle units primarily concerns morphological differences of homologous muscle units rather than divergence in the developmental splitting pattern. The most notable examples are the *m. abductor indicis longus* and the *m. extensor digitorum communis longus*, whose morphological variation may reflect underlying phylogenetic divergence and/or functional specializations related to species-specific locomotor or behavioral adaptations.

CONSERVATION

Artificial habitats: lifeline or last straw for European amphibians - Quantifying the importance of artificial habitats for amphibians in Europe's anthropogenic landscapes

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Abstract

Habitat loss related to land-use change is generally considered a primary driver of amphibian biodiversity loss. Paradoxically, in Europe many amphibian species, including threatened ones, can persist and even thrive in heavily modified landscapes. However, a comprehensive understanding of the contribution of artificial habitats to amphibian conservation across Europe is lacking. Here, we employed Area of Habitat (AOH) maps, which delineate a species maximum potential habitat to quantify the total habitat available to 73 amphibian species in Europe and infer the proportion of artificial habitat for each.

Our analysis revealed a significant negative correlation between the proportion of artificial habitat and the IUCN threat status of amphibians, suggesting that those amphibian species which are less able to utilize artificial habitats tend to be more threatened. Artificial habitats comprised on average only 37.6% to 26.3% of a species total AOH. Nevertheless, we identified a notable subset of species (20 to 35), several of which are threatened, that exhibited a strong reliance on artificial habitats (comprising more than 50% of their AOH). Frequently these species are poor competitors and are thus reliant on temporary waterbodies for reproduction which are part of dynamic habitat networks. These findings highlight that amphibian diversity in Europe can only be safeguarded by combining habitat conservation and restoration with innovative strategies that consider the varied importance of artificial habitats.

CONSERVATION

The Frogs in My Backyard: Investigating Amphibians Using Mini-Ponds and Citizen Science in Austria

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Abstract

Amphibians are among the most endangered animal groups worldwide, with habitat loss due to land use changes posing a significant threat to their survival. The loss of

small, early-succession water bodies is particularly critical for pioneer species that depend on them for reproduction. To address this issue, the establishment of man-made ponds has emerged as a promising conservation strategy to mitigate habitat loss and support amphibians. The AmphiBiom project (2024–2025) applies a citizen science approach to study amphibian occurrences across Austria, including areas that are difficult for researchers to access, such as private gardens. In 2024, we distributed 302 polyethylene mini-ponds (120 × 90 × 40 cm) to selected citizen scientists to place them strategically within the distribution range of the green toad (*Bufo viridis*), our target species. Citizen scientists collected data bi-weekly from March to August 2024 using the specially developed mobile phone application AmphiApp. Besides obtaining photographs and sounds of amphibians, citizen scientists sampled aquatic invertebrates from their mini-ponds, preserved them in ethanol and returned them to the research team for detailed analysis. Our team identified and documented species composition during the ecological succession of the mini-ponds. In 2024, the first year of the experiment, *B. viridis* and at least 12 other amphibian taxa were recorded at 38% of the sites monitored. Additionally, signs of amphibian reproduction were observed in 10% of the mini-ponds. These first results are promising, and we expect that more amphibians will discover the mini-ponds for reproduction over time. Our findings demonstrate that even small man-made water bodies may aid in stabilizing amphibian populations, offering a scalable and effective conservation measure to counteract habitat loss.

ECOLOGY

What is a slow-worm's tail for?**Nicholas Smith**Corresponding author: nickanguis@yahoo.co.uk**Abstract**

Autotomy, or deliberate shedding of the tail, is common in lizards as a predator escape strategy. Many European lizards regrow the tail quickly to regain normal locomotion. In many lizards the tail is involved in fat storage for reproduction and hibernation so that autotomy can involve a reproductive cost.

Slow-worms (*Anguis*) practice autotomy, but tail regrowth is very slow and never complete. Autotomy has often been seen in cost-benefit terms. There is little cost in a slow-worm's small regrowth, so we need to ask if there is another, less obvious one.

Slow-worms were sampled by mark-recapture and removal at sites in Hampshire and Dorset, southern England. Length, sex and weight of nearly 6000 animals and data for > 300 litters were also recorded.

These data were used to assess tail growth relative to snout-vent length (SVL). At birth the tail is a little shorter than the SVL. It grows at a faster rate than the SVL until sexual maturity at c. 130 mm and about 115 % of the SVL. Relative growth then slowed, the female's more so than the male's. In larger sized adults (190+ mm SVL) a male's complete tail was about seven mm longer than a female's. The sexes had similar rates of autotomy. Only about 20% of larger animals retained complete tails.

Typically there were four to ten young in each litter. Females with autotomized tails had significantly fewer ($p < 0.05$) young than those with complete tails of the same SVL. This difference increased with female SVL. Females with SVL > 180 mm with autotomized tails had two or three fewer young than those with complete tails. Size or condition of the young was not influenced by the length of the tail. At a population level, it was estimated that five percent fewer young were born, which could be an important cost.

ECOLOGY

Frogs versus fish – experimental predation of native and non-native fish on early life stages of six European anuran species

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Abstract

While many amphibian species thrive in temporal water bodies devoid of fish, coexistence is frequent and complex. Introduction of non-native fish may hamper amphibian survival even further, both as eggs and as larvae. This study investigated the predatory impact of eight fish species (five native, three non-native) on the larvae and tadpoles of seven different anuran species (*Alytes obstetricans*, *Bufo bufo*, *Hyla arborea*, *Pelobates fuscus*, *Pelophylax kl. esculentus*, *Rana temporaria*). Results reveal marked differences both in the predatory behaviour of the fish and in the susceptibility of the amphibian larvae, highlighting potential species-specific interactions and seasonal patterns. Non-native species as a whole demonstrated higher predation rates, suggesting they may pose a significant threat to amphibian populations. These findings contribute to a better understanding of fish–amphibian interactions and have implications for amphibian conservation and freshwater management.

EVOLUTION

Nuptial pads of *Xenopus* frogs depend on a keratinization program that is homologous to the cornification of mammalian skin appendages

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Abstract

Hair keratins are the main protein components of cornified appendages in mammals. Recently, we have shown that homologs of hair keratins are expressed in the cornified claws of *Xenopus* frogs. Here, we tested the hypothesis that these keratins have additional functions in other cornified structures of amphibians. Histological analysis of nuptial pads on the clawless forelimbs of *Xenopus laevis* and *Xenopus tropicalis* demonstrated the presence of microscopic cornified spines. Transcriptomic and proteomic analysis showed that *krt59*, a homolog of type II hair keratins, is expressed in nuptial pads. This was further confirmed by in situ mRNA hybridisation, which demonstrated that *krt59* is specifically expressed in the cornified spines of nuptial pads. Surprisingly, the expression of *krt59* was not accompanied by the expression of a type I hair keratin homolog but by the expression of other type I keratins. The transcription factor *Hoxc13*, which regulates hair keratins in mammals and their homologs in the claws of *Xenopus* frogs, was significantly elevated in nuptial pads as compared to the soft skin of frogs. CRISPR-Cas9-mediated inactivation of *Hoxc13* diminished the expression of *krt59* and the cornification of nuptial pads. We conclude that nuptial pads are a second site of *Hoxc13*-dependent hard cornification in frogs with hair keratin expression being only partly conserved. Our results point to partial conservation of epithelial cornification mechanisms across tetrapods and amphibian-specific adaptations of an ancestral program for cell differentiation in skin appendages.

CONSERVATION

Engaging Communities to Detect *Hyla arborea*: Four Years of Citizen Science Surveys Through the REGAlnica Event in Southwestern Slovenia

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Abstract

Recent data on the presence of the European tree frog (*Hyla arborea*) in southwestern Slovenia were limited before May 2022, despite amphibian surveys conducted in 2020 and 2021. In this region, the species primarily breeds in small and often temporary ponds that have declined due to abandonment, desiccation, and successional overgrowth, or have become unsuitable due to fish introductions. The species is often overlooked in daytime surveys; however, it is more easily detected with acoustic monitoring during breeding season evenings. To obtain more comprehensive data, we organized a public event called “REGAlnica,” conducted one evening each May from 2022 onward. Using a citizen science approach, we organized volunteers surveying potential breeding habitats and recording species presence by acoustic (detection of calls) or visual confirmation. The event started with 51 volunteers in 2022, reaching its highest participation so far in 2024 with 78 volunteers. Volunteers were trained to identify the species by its vocalizations and were provided with clear protocols before the events. Survey sites were selected from two databases: the Center for Cartography of Fauna and Flora’s and the University of Primorska’s pond database. Most monitored sites had previous records of the species. Over the first four years, a total of 193 unique sites (ponds or other water bodies) were visited (115 in 2022, 110 in 2023, 137

in 2024, 141 in 2025), with 61 sites revisited annually. In total, *Hyla arborea* was confirmed at 57 sites (29.5% of all sites surveyed). It was confirmed at 29 sites (25,2%) in 2022, 36 sites (32,7%) in 2023, 17 sites (12,4%) in 2024, and 25 sites (17,7%) in 2025. Compared to past records the combined results suggest a possible decline in *Hyla arborea* presence in the study area. Low temperatures at some sites, particularly pronounced in 2024, affected calling activity and detection, highlighting the importance of systematic, repeated monitoring.

CONSERVATION

A Multi-Modelling Approach for Informing the Conservation of a Cold-Adapted Terrestrial Amphibian in the Face of Climate Change

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Abstract

Species Distribution Models (SDMs) are a widely used tool to understand species' response to climate change. Here, we used intraspecific SDMs to model climate change impacts on two lineages of alpine salamanders that show a strong phylogeographic structure (*Salamandra atra atra* from the Alps and *Salamandra atra prenzensis* from the Dinarides); we also included one species-level model (*Salamandra atra* as undifferentiated entity). Predictions incorporated uncertainties arising from occurrence precision and modelling choices by using species' occurrences from a carefully curated database and by applying eight modelling techniques combined with 24 GCMs and two RCP scenarios. We used model consensus layers to define the impact of climate change on each modelling unit. For *S. a. atra*, under RCP2.6 scenario, we detected a 3% increase in the extent of climatically suitable areas in the Alps (although with low model agreement), yet a 6% decrease under the RCP8.5 scenario. For *S. a. prenzensis*, we detected a decrease in the extent of climatically suitable areas in the Dinarides between 7% and 45% (RCP2.6 and RCP8.5, respectively). The SDM predictions for the entire species were most in line with those of the *S. a. atra* model. Although climate variables were not equally strong in predicting

the climatic suitability for both lineages, the minimum temperature of the coldest month revealed an important predictor for the entire species, with clear decreases in suitability towards higher temperature. In conclusion, all models reveal a considerable decrease in climate suitability throughout most range of *S. atra*, which is particularly visible in the Dinarides. We highlight the importance of considering intraspecific variation when modelling climate change impacts on geographically differentiated species whose populations vary in their climatic niche. We provide our study results (occurrence data and maps) via a web application that can be useful for guiding conservation efforts

CONSERVATION

Urban habitats and Amphibian Vulnerability: A Study of Road Mortality and Mitigation of Amphibian Mortality in Delhi-NCR, India

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Abstract

Urbanization-driven habitat fragmentation poses a major threat to amphibians, especially in rapidly expanding cityscapes like Delhi-NCR, where road infrastructure intersects critical breeding and foraging habitats. Amphibians, characterized by limited dispersal ability, strong site fidelity, and seasonal breeding migrations, are especially susceptible to road mortality. Between 2020 to 2023, a field study was conducted across urban and peri-urban sites in Delhi-NCR to assess species diversity, identify breeding habitats, document roadkill patterns, and implement mitigation strategies. Eight species of amphibians were recorded – *Microhyla nilphamariensis*, *Uperodon systoma*, *Duttaphrynus melanostictus*, *D. stomaticus*, *Minervarya pierrei*, *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis*, and *Sphaerotheca maskeyi*. Many of these were confined to fragmented habitats with low population densities. Roadkill incidents peaked during monsoon and post-rainfall periods, primarily affecting breeding adults. Based on seasonal patterns and habitat associations, mitigation strategies were implemented at selected high-risk sites. One such site, prone to waterlogging that attracted amphibians to breed directly on the road surface, was modified through the installation of a well-designed water channel that effectively diverted standing water off the road. This intervention resulted in a marked decline in amphibian road mortality. Developed in collaboration with local residents and civic authorities, this site-specific solution, offers a viable alternative strategy to conventional culvert systems, especially in areas where water accumulation is a key driver of amphibian roadkill. Additionally, species-level bioacoustics data were collected to support ongoing non-invasive monitoring. Project outcomes were documented in an awareness film co-produced with stakeholders, highlighting the success of participatory conservation in mitigating amphibian road mortality in urban environments.

ETHOLOGY

In situ observations of breeding behaviour and context specific vocalization in the largest frog from India, *Hoplobatrachus tigerinus*

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Abstract

Hoplobatrachus tigerinus, the Indian bullfrog, is a large semi-aquatic microglossid amphibian commonly found across the Indian subcontinent. It predominantly occupies ephemeral water bodies, where it functions as both predator and prey. Despite its wide distribution, many aspects of its reproductive ecology and acoustic behaviour remain poorly understood, especially in light of escalating anthropogenic threats such as habitat degradation, pollution, and overexploitation. This study presents the first comprehensive documentation of the breeding behaviour of *H. tigerinus*, along with the description of its context-specific vocalizations and a preliminary description of the female call. Field investigations were conducted over two consecutive monsoon seasons, in 2022 and 2023, in the semi-urban landscape of Gurgaon, India. Observations revealed explosive breeding aggregations with a male-biased sex ratio and asynchronous female arrival. Field observations and sound recordings were made at midnight when the species breeding activity and male vocalization were at their peak. After courtship, the pairs were observed in axillary amplexus, during which inter-male competition was also noted. The entire courtship and amplexus lasted for about 120 minutes, which was followed by multiple spawning events. The species was observed to produce three distinct vocalizations: advertisement calls, amplexus calls (pre and post-oviposition), and female calls (low-pitched pigeon-like “gutrr-goo”). Males produced pulsatile calls lasting approximately 199.2 ms, comprising 17 pulses at a rate of 87.1 pulses/s, with a dominant frequency peaking at 1.2 kHz. Several previously unreported call types were identified, suggesting

a diverse vocal repertoire strongly correlated with breeding activity. We further compared these calls with *H. crassus*, a congeneric species. These findings provide foundational insights into species-specific communication, aiding conservation and behavioral ecology.

CONSERVATION

Characterizing invasive hybridization between two deeply diverged newt species using genome-wide data

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Abstract

Invasive species contribute to biodiversity loss. A particularly insidious mechanism is genetic pollution: anthropogenic hybridization between native and introduced species. This process targets biodiversity at the genetic level, effectively overwriting parts of the native gene pool. Over a decade ago, the non-native Anatolian crested newt (*Triturus anatolicus*) was introduced into Montnegre i el Corredor Natural Park (Catalonia, Spain). It began hybridizing uncontrollably with the native marbled newt (*T. marmoratus*), prompting eradication. We use target sequence capture (NewtCap) and mtDNA barcoding to assess the frequency, composition and fertility of these hybrids. To identify the source of the introduction, we compared the invasive genotypes with a reference dataset spanning the native range of *T. anatolicus* in Turkey. Our results confirmed the presence of F1 hybrids and a relatively high frequency of backcrosses compared to the natural hybrid zone between *T. marmoratus* and the northern crested newt (*T. cristatus*). We detected strong directionality in the hybridization, with F1 hybrids almost exclusively having a *T. anatolicus* mother. All later-generation hybrids were backcrosses towards *T. marmoratus*. The origin of the invasive *T. anatolicus* population was traced back to Lake Abant in western Asiatic Turkey. Backcrossing raises the possibility of adaptive alleles entering the native *T. marmoratus* gene pool. Our study highlights the need for gene-wide monitoring to inform management actions.

MORPHOLOGY

Modularity in four morphotypes of the salamandrid skull – conserved or not?

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Abstract

The salamandrid cranial morphology is largely conserved. A series of four recognized skull morphotypes ranges from a complete frontosquamosal (FS) arch (A), a partially reduced (B), a largely reduced (C) to a completely reduced FS arch (D). Character state changes as here studied are found scattered over the salamandrid phylogeny. The species investigated for skull static modularity are *Paramesotriton hongkongensis* (A), *Calotriton asper* (B), *Ichthyosaura alpestris* (debatedly *Mesotriton alpestris*) (C), *Salamandra salamandra* and *Triturus cristatus* (D). The static allometry is only significant in *C. asper* and *I. alpestris*, so allometric corrections were not applied for the entire sample. The four hypotheses of modularity based on developmental and/or functional criteria here considered are: i) somite derived occipital region vs. the remainder of the skull, ii) idem for the naso-orbito-otic, iii) maxilopalatal vs. skull roof, and iv) the thyroxine induced development hypothesis. The modularity hypothesis with the strongest signal is the naso-orbito-otic one, whereas *T. cristatus* has the strongest signal for the maxilopalatal vs. skull roof hypothesis. The naso-orbito-otic hypothesis is functional/developmental, related to the growth of the brain and the sensory organ capsules, whereas the maxilopalatal vs skull roof hypothesis is functional, related to the feeding apparatus. Static modularity in the salamandrids appears to be conserved, mostly influenced by functional constraints while the role of allometry in shaping modularity patterns remains somewhat inconclusive. Future studies should include more species and explore the evolutionary component of the skull modularity and morphological integration.

CONSERVATION

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

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Abstract

While a decline of great crested newt (*Triturus cristatus*) populations is well documented in some regions of Europe, its status in northeastern France is not precisely known. A long-term monitoring program has started in 2021 in northeastern France in order to assess its abundance variation over time through a randomised sample of 172 ponds within nine natural regions where the species occurs. Two different statistical models have been confronted back-to-back, one solely based on occurrences (multi-year occupancy model), and another integrating multiple counts at each site (N-mixture model), with the aim of obtaining two indicators to track trends of the species at a local and global scale. In 2021 and 2024, we found a mean occupancy rate of 0.67 associated with a detection probability of 0.73 with the first model, and a total population estimated at 1850 newts with a maximum detection probability of 0.32 with the second model. Distance to the nearest occupied ponds negatively influences occupancy, whereas it positively influences abundance within ponds. Bigger and isolated ponds seem to bear the highest density of newts, whereas in metapopulation-structured systems, abundance in ponds is lower but there are more occupied ponds within the pond network.

We discuss the core differences between the two approaches, as well as how they enabled to identify putative metapopulations. This comparative study should allow to improve the efficiency of the long-term monitoring of this endangered species as well as providing insights for conservation of pond networks.

CONSERVATION

The Natterjack Toad in Northern Belgium: Genes, Habitat & Translocation

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Abstract

The natterjack toad (*Epidalea calamita*) is threatened in Flanders, Belgium, primarily due to habitat loss and fragmentation. This study assesses the genetic structure, diversity, and connectivity of natterjack populations across the region, integrating these insights with habitat quality and population viability data to guide future translocations.

We sampled 42 populations and analyzed genetic variation using microsatellite markers. Field surveys estimated population densities and evaluated habitat conditions. Additionally, we reviewed past translocation efforts and identified potential reintroduction sites.

Results reveal that many populations are small, isolated, and genetically impoverished, with limited connectivity among clusters. Conversely, a few core populations maintain high genetic diversity and form connected metapopulations, these are confined to a few coastal dunes and larger heathlands.

These findings emphasize the urgent need for targeted conservation measures, including habitat restoration and enhanced connectivity. Furthermore, we discuss the challenges of translocations within this ecological context.

CONSERVATION

Role of CITES Scientific Authorities in conservation: new developments

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Abstract

The European Union (EU) is an important importer of wildlife and has a responsibility for the conservation of respective species. CITES (Convention of international trade in endangered species of wild fauna and flora) aims to ensure a legal and sustainable trade in listed species. The presentation will provide an overview on the role of CITES Scientific Authorities in wildlife conservation. In particular recent research activities of the and projects German Scientific Authority (Federal Agency for Nature Conservation, BfN) will be presented with a focus on a) listing species in the CITES Appendices also in view of the upcoming CITES CoP in November 2025, as well as b) implementation of listings. The latter will focus on the challenge to verify the captive origin of live reptile and amphibian species in trade, which gained increasing importance among CITES authorities worldwide. Currently, trade in CITES listed live reptiles and amphibians mainly involves animals declared as captive-bred. While legal and sustainable trade in captive-bred animals can contribute to species conservation, trade in wild-caught animals that are falsely declared as captive-bred to circumvent trade restrictions can be very harmful to wild populations.

The BfN therefore has for the third time commissioned the compilation of guidance, including comprehensive data on the reproduction, biology, and husbandry requirements for the species most recently listed in CITES Appendices I and II. These data were collected through interview surveys and literature research and compiled by the DGHT. In addition, an assessment of the difficulty and complexity of keeping and breeding these species in captivity was provided. The most recent and third guidance of its kind was just finalized and aims to support national and international conservation authorities in verifying the plausibility of breeding claims, thereby improving the implementation of the CITES convention and helping to curb illegal trade.

SYSTEMATICS

Phylogeny of the racer genus *Platyceps* (Squamata: Colubridae) based on different genetic approaches**Doubravka Velenská**

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Abstract

For a long time, the phylogeny of the genus *Platyceps* has remained a mystery. Previous genetic studies typically contained just a few species from the otherwise species-rich genus (with 33 currently recognized species) and served as a by-product of much larger-scale analyses. Firstly, we reconstructed for the first time a robust phylogenetic tree of *Platyceps* based on 169 samples representing 19 species (with 115 own samples) based on Sanger sequencing of 4 mitochondrial (12S rRNA, cyt-b, ND4 + tRNA, coi) and 2 nuclear (c-mos, NT3) markers. These results allowed us to comprehensively elucidate the phylogeny of the genus and the relationships between the species and assess the biogeographic origins of the genus by conducting a historical biogeographic analysis. Moreover, it showed us that more detailed studies need to be carried out to resolve the taxonomy of several of the species. To support the results of the analysis of the Sanger data, we assembled another dataset, this time using the ultra-conserved elements (UCE) to reliably reconstruct the relationships between the three main clades of the genus. To shed more light on the intraspecific structure within one of the most broadly distributed species *Platyceps rhodorachis* we used another approach – ddRAD sequencing, which is ideal to resolve population-level relationships and species demographic history. Finally, from a taxonomic perspective, it was crucial to evaluate the status of taxa known only from the type series, some of which were described more than 100 years ago with the type specimens stored in formalin. For this analysis, we used the ancientDNA analysis methods with sequencing the whole mitochondrion and a few nuclear markers. In all, we applied several analytical approaches using different genetic/genomic methods to be able to robustly reconstruct the *Platyceps* phylogeny at different phylogenetic and taxonomic scales.

CONSERVATION

Criteria for and selection of Important Herpetofauna Areas in Britain and beyond

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Abstract

Approaches for the identification of areas important for biodiversity have already been widely implemented and accepted across several taxonomic groups including birds, butterflies, plants, and invertebrates. Both the process of identifying important areas, and the subsequently identified areas themselves deliver benefits for biodiversity. These include providing evidence towards site safeguarding, protection and designation, offering a point of focus for policy and on the ground conservation efforts, and highlighting threatened biodiversity to wider audiences. To date however, no single approach for important area identification has been formally agreed and adopted for amphibian and reptile species within Great Britain or at the European scale.

Here, I review potential criteria for the spatial identification and prioritisation of Nationally important areas for herpetofauna (Important Herpetofauna Areas - IHAs) and demonstrate their application in Great Britain. Using available species occurrence data and the national Red List assessment of amphibians and reptiles in Great Britain, I accounted for the distribution of Critically Endangered species, Threatened species, and Nationally Important Assemblages (as identified by species richness). Based on these criteria I identified 655 10km grids (hectads) of interest, including 210 Primary IHA hectads that support both threatened (including Endangered) species, and Important Assemblages. These Primary IHAs should be the focus of further steps to define ecologically relevant site boundaries, and to assess additional requirements for designation and protection. I further discuss the possibilities, next steps, and challenges of applying IHAs at the European level, and for reviewing IHAs at the local level in Great Britain. These findings may be useful for other countries seeking to identify areas of national importance, and for European-scale assessments.

CONSERVATION

Effectiveness of wetland restoration on amphibians in a drying grassland habitat losing its breeding sites

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Abstract

Amphibians are one of the most threatened taxa globally, partly due to habitat loss, including the loss of suitable breeding sites. This problem is severe in the Hungarian Great Plain (Central Europe), which has lost the majority of its wetlands due to excessive draining. The remaining wetlands are further threatened by climate change, as the increasing intensity of summer droughts significantly decreases the length of the hydroperiod, threatening wetland communities. To mitigate the impacts of climate change on wetland communities, 23 wetland pools were restored at 13 sites in the „Felső-kiskunsági turjánvidék” Natura 2000 site in Central Hungary. In this study we compared amphibian occupancy in restored and near-natural wetlands and artificial ditches; tested the effects of hay and mud transfer on amphibian occupancy and assessed the habitat requirements of the different species. We surveyed amphibians at all 13 restoration sites, 6 nonrestored wetlands as controls and 8 ditches in the spring of 2024. We used multispecies occupancy models to estimate occupancy in the study sites and investigate the effects of different wetland parameters and restoration techniques on amphibian occupancy. Amphibian occupancy was higher in restored wetlands compared to nonrestored controls, with both hay and mud transfer having positive effects on amphibian occupancy. While the different species had different habitat preferences, generally water depth had a positive effect on amphibian occupancy. Our study highlights the importance of wetland restorations for amphibians in drying landscapes and provides insight into the habitat preferences of multiple amphibian species.

EVOLUTION

Unravelling an evolutionary mystery: the balanced lethal system in *Triturus* newts

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Abstract

A balanced lethal system is a deadly hereditary disease that causes the loss of fifty percent of offspring every generation. The most infamous example concerns the chromosome 1 syndrome affecting the salamander genus *Triturus*: the crested and marbled newts. All adults possess two forms of chromosome 1, known as 1A and 1B. Yet, according to the rules of Mendelian inheritance, 50% of offspring are homozygous, possessing either 1A or 1B twice (and lacking the other version). Such individuals die roughly halfway embryological development – 50% of *Triturus* eggs never hatch! We show that *Triturus*' 1A and 1B (1) are mostly conserved across the genus; (2) form two distinct, consecutive blocks in the genomes of both *Lissotriton* and *Pleurodeles* newts; and (3) share a phylogenetic relationship that is distinct from that of the rest of the *Triturus* genome.

OTHER

Current state, prospectives and challenges of herpetology in Ukraine

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Abstract

Current situation with herpetology in Ukraine resulting from an interaction of number of factors. Political situation, war, depopulation and brain drain, lack of funding and dynamic academic landscape influence the volume and level of research. The majority of research are devoted to biodiversity monitoring and its conservation in times of global climate and biota changes, documentation of destruction and fragmentation of habitats. Situation with herpetology in Ukraine is a part of bigger picture of lack of biodiversity specialists and research initiatives globally. We call for a wider engagement of citizen scientists to monitoring of current state of populations of amphibians and reptiles, implementation of DNA based monitoring (eDNA, DNA barcoding), surveillance of emerging and known pathogens, all within targeted funding of strategic initiatives of science support, which is still need to be developed. Examples of challenges and current project in Ukraine will be given.

CONSERVATION

Fish like amphibians, but amphibians don't like (cray)fish! An overview of best and worse practices in the Netherlands

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Abstract

In fens and ponds fish are able to live under natural circumstances, mostly temporarily. So amphibians have to deal with natural predators like fish and therefore they lay a quite large number of eggs or bring larvae to birth. So, why are we worried about this relationship?

In the Netherlands we have been monitoring amphibian populations for more than three decades. Sometimes threatened species like the midwife toad (*Pelobates fuscus*) suffered from a sudden decrease in numbers. Such was the case in the 'Rauwven', a natural fen, in the early 2000s. It appeared that introduction of Pumpkinseed (*Lepomis gibbosus*) lead to a disaster. During succesfull eradication of all fish, the pond was dried artificially and a sandlayer was brought in to make the fen more shallow. If and how midwife toad restored I will tell you at the workshop. Pumpkinseed was introduced in 1905 in the Netherlands, before the year 2000 there were 91 of 5km gridcells known where they occurred and between 2000 and 2025 at 477 gridcells. To stop this increase we decided to inform the pet trade and try to bann the selling of invasive alien fish species at gardening shops. More cases will be discussed as well as failures.

The latest problem for amphibians is a tsunami of exotic crayfish species, which is not only a serious problem in ditches and ponds in meadow peatland, but also in isolated ponds in the drier eastern parts of the Netherlands. Our experiences may help the further development of prevention of (cray)fish in ponds and ways to eliminate them, and may help a further discussion and exchange of experiences during this workshop.

Symposium

Herp-omics: a genomic perspective on earth's herpetofaunal diversity

Organisers: Alice Petzold & Carolin Dittrich



EVOLUTION

Using Off-target Reads for De Novo Investigation of Mitochondrial Evolution in Cophyline Frogs (Microhylidae)

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Abstract

Hybrid-enrichment has gained popularity as a third-generation sequencing method targeting potentially thousands of markers. Off-target reads, dominantly comprising mitochondrial DNA due to its large copy number, have the potential to yield valuable additional data. We performed successful de novo assemblies of structurally unique mitochondrial genomes using off-target reads generated by hybrid-enrichment target capture performed on 165 frog specimens. As de novo genome assembly algorithms are designed with whole genome sequencing (WGS) data in mind, the gaps inherent to reduced representation sequencing methods like target capture can hinder their effectiveness. To address this, we developed a seed exploration method to optimize seed selection for seed-based de novo assembly. Our methods produced circularized mitochondrial genome assemblies covering the generic-level diversity of the Microhylid frog subfamily Cophylinae. Cophyline frogs are found exclusively on Madagascar and exhibit great taxonomic diversity, with 117 currently described species in 9 genera. Our mitochondrial genome assemblies have facilitated the creation of a new, well-resolved phylogeny that supports generic relationships previously reported in literature; and reveals the existence of derived reorganization of mitochondrial structure that arose in the ancestor of three genera: Stumpffia, Anilany, and Rhombophryne, while independently confirming the novel mitochondrial genome structure previously reported for Anilany based on WGS data. Furthermore, we applied Bayesian dating methods to estimate the evolutionary timing of mitochondrial gene rearrangement, and used algorithmic methods to calculate the most parsimonious mechanisms for the observed rearrangements. Our results indicate off-target reads from target capture data can be used to confidently reconstruct mitochondrial genomes with variable structure, and provide a guideline for optimizing the implementation of de novo assembly.

SYSTEMATICS

Phylogeographic and genomic insights unveil the evolutionary history and post-glacial recolonization routes of the Palmate Newt (*Lissotriton helveticus*) into Europe

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Abstract

Quaternary glacial cycles have been key drivers of diversification for Holarctic species, promoting divergence, isolation, and extinction processes in numerous taxa. These cycles facilitated evolutionary radiations in some groups but also erased much of the evolutionary history of species with northern origins. Here, we investigate the

evolutionary and phylogeographic history of the Palmate Newt (*Lissotriton helveticus*), a widespread species in post-glacial ecosystems in Western Europe. We generate genome-wide ddRADseq for 205 individuals from 51 populations across the species range and reconstruct its phylogeographic and demographic history, assess population structure, and characterize ecological paleo-niches for the species at different climatic periods. Results identify several distinct lineages exhibiting strong genetic differentiation, primarily driven by geographic barriers and isolation in historical refugia with admixture in transition zones. Phylogeographic reconstructions suggest that the main glacial refugium for *L. helveticus* was most likely located in northern Iberia. Two main dispersal routes were identified: one extending eastward through the Ebro River Basin and, a second, following a north-eastward pathway across the Pyrenees and into Europe. We specifically pinpoint the origin of Europe's recolonization route to a specific set of localities surrounding Andorra, where *L. helveticus* probably expanded along tributaries to the Garonne River into southern and western France over warm periods. By integrating genomic, geographic, and paleoclimatic data, this study provides an in-depth understanding of how climate shaped the evolutionary history of this temperate species and reinforces the importance of waterways for amphibian dispersal dynamics.

CONSERVATION

Clinging on the brink: Conservation genomics of the Critically Endangered Emirati Leaf-toed Gecko (*Asaccus caudivolvulus*)

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Abstract

The Emirati leaf-toed gecko (*Asaccus caudivolvulus*) is the only endemic vertebrate of the United Arab Emirates and is currently listed as Critically Endangered. Rapid coastal development in the UAE has severely fragmented and degraded its natural habitat, raising concerns about its persistence. In this study, we generated a chromosome-level reference genome for *A. caudivolvulus* and resequenced whole genomes of 23 individuals representing all Arabian and selected Iranian *Asaccus* species. We located five remnant populations of *A. caudivolvulus* and analyzed their genomic status. Results show the species harbors the lowest genetic diversity of the group and exhibits alarming levels of recent inbreeding in heavily developed areas, as reflected in a >50% increase in long runs of homozygosity within just nine years in one of the populations. Demographic analyses reveal a continuous decline in effective population size over the past 1.5 million years. Population structure analyses show genetic isolation between localities, with no recent gene flow. Altogether, these results indicate

that *A. caudivolvulus* faces a high risk of extinction due to genetic erosion and ongoing habitat loss. Immediate conservation actions, including habitat protection and genetic management, are urgently needed to prevent the loss of this unique vertebrate lineage.

CONSERVATION

To see the bigger picture: using genomic tools for the conservation of neotropical amphibians facing emerging disease threats

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Abstract

Amphibians are among the most threatened species, facing increasing global and local pressures. Effective conservation strategies must therefore integrate knowledge of species biology, demography, and the ecosystems they inhabit. Emerging diseases such as chytridiomycosis represent major threats, yet our understanding of their dynamics, particularly in relation to host demography and climate-driven environmental factors, remains limited.

In the amazonian rainforest, chytrid fungus continue to spread, impacting species such as *Dendrobates tinctorius*, a poison frog endemic to the eastern Guiana Shield. Recognized as a sentinel species for chytrid monitoring, *D. tinctorius* exhibits a strikingly variable aposematic coloration contributing to strong phenotypic structuring. This richness offers a valuable window into many evolutionary processes. Preserving these populations is therefore essential—not only for maintaining biodiversity, but also for deepening our understanding of the evolutionary dynamics shaping life in the Neotropics.

Long-term monitoring of chytrid prevalence at multiple sites has revealed strong fluctuations, likely driven by environmental variables. High-throughput genomic sequencing of 192 individuals from across the region has uncovered fine-scale genetic structure and distinct genetic clusters, reflecting a complex demographic history. *Fst* values indicate high genetic differentiation between populations, yet with unexpectedly high levels of heterozygosity. Species distribution models suggest limited connectivity among populations emphasizing their vulnerability to pathogen outbreaks.

Our next step involves third-generation sequencing to characterize metagenome of individuals across populations and identify drivers of pathogen community diversity. Altogether, these findings will inform the development of robust, science-based conservation strategies to safeguard the amphibian of the Neotropics against the growing threat of emerging diseases.

ECOLOGY

Dietary Patterns of Marine Iguanas Across the Galápagos Archipelago Revealed by DNA Metabarcoding

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Abstract

The Galápagos marine iguana (*Amblyrhynchus cristatus*) is the world's only marine-adapted lizard and an iconic example of ecological specialization. Although long known to feed primarily on marine algae, the spatial variation and fine-scale composition of their diet across the archipelago remain poorly understood. In our study, we applied high-throughput DNA metabarcoding to faecal samples collected from marine iguana populations across 13 islands, encompassing representatives of all recognized subspecies. We used universal eukaryotic 18S rRNA markers to capture a broad snapshot of dietary diversity, and complemented this with a targeted set of *rbcl* primers specifically designed to improve resolution of red algae, the iguanas' primary dietary group. This dual-marker strategy enabled the detection of algal taxa as well as other dietary components, revealing fine-scale spatial patterns and site-specific variation in diet. Our study provides the first archipelago-wide molecular assessment of marine iguana foraging ecology, offering new insights into the flexibility and constraints of herbivory in this emblematic reptile. Our results also highlight the power of non-invasive metabarcoding to uncover ecological interactions and dietary variation in a highly specialized marine herbivore across the diverse and isolated environments of the Galápagos Islands.

CONSERVATION

Introduction to the Amphibian Genomics Consortium

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Abstract

The Amphibian Genomics Consortium (AGC) is an international initiative that brings together individuals with a shared interest in utilising genomic tools to enhance our knowledge of the ecology, evolution and conservation of amphibians. Due to the mounting threats faced by this group, the necessity for amphibian genomic resources is becoming increasingly pressing. Especially, since this field has facilitated a more profound understanding of ontogenetic diversity, tissue regeneration, diverse life history and reproductive modes, anti-predator strategies, resilience and adaptive responses to environmental changes. Furthermore, the complex amphibian genomes serve as indispensable models for the study of broad genomic traits, including evolutionary genome expansions and contractions, given that they exhibit the widest range of genome sizes among all animal taxa and possess multiple mechanisms of genetic sex determination.

Initiated in March 2023, the AGC is building upon the efforts of existing genomics consortia, including the Vertebrate Genomes Project (VGP), the Earth BioGenome Project (EBP), and the Darwin Tree of Life (DTOL) project and aims to support amphibian-specific sequencing projects and other genomics-driven research and applications. The field of amphibian genome sequencing has been comparatively underdeveloped in comparison with that of other vertebrates. This is primarily due to the challenges inherent in assembling their large, repeat-rich genomes, as well as the relative lack of societal support. This presentation will outline the AGC's objectives and scope, focusing on current developments and achievements by its members and board. Additionally, an overview of published papers, ongoing surveys and participation opportunities will be provided.

ECOLOGY

A comparison of fire salamander (*Salamandra salamandra*) dermal microbiota along an anthropogenic divide through whole genome shotgun sequencing

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Abstract

Amphibian dermal microbiota has received much attention with the hypothesis that certain species may confer resistance to fungal chytrid infections. Lower rates of chytrid fungi have been observed among urban habitats, however, the number of studies on urban system are few and the causal factors uncertain. The impacts of urban soil and water contamination on microbiota have also received limited attention among amphibians, and while characterization of amphibian microbiota by targeted 16S rRNA sequencing has indicated community shifts in microbiome composition, we sought to apply high throughput shotgun sequencing to assemble microbial genomes to improve taxonomic resolution and infer functional gene information. Our focal species for this study are fire salamanders, *Salamandra salamandra*, sampled from within the urban core and rural periphery of Porto, Portugal. Despite only a small proportion of total generated data were mapped to bacteria, over a hundred microbial-assembled genomes were assembled, most of which are likely new species. Differences between urban and rural habitats were observed in regard to species composition and phylogenetic diversity, along with habitat-specific prokaryotic lineages. Further investigation into functional variation between microbiota assemblages is ongoing, with a focus on ecotoxicology.

BIOGEOGRAPHY

A Biogeographic Overview of Fire-Bellied Toad Diversity

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Abstract

The genetic and phenotypic diversity of species is strongly shaped by their biogeographic history, through processes such as divergence, hybridization, demographic dynamics, local adaptation, and phenotypic plasticity, all interacting with environmental fluctuations. In this presentation, I explore the biogeographic factors underlying patterns of diversity in fire-bellied toads of the *Bombina* subgenus—a well-studied group currently comprising three species: two in Europe (*B. bombina* and *B. variegata*) and one in Asia (*B. orientalis*). To this end, we combined phylogeographic approaches (mitochondrial barcoding and genomic analyses) with morphological, bioacoustic, bioclimatic, and coloration data across several thousand specimens. Molecular data reveal high intraspecific genetic diversity, signatures of both ancient and recent hybridization, and post-glacial expansions, highlighting the need for taxonomic revisions. Phenotypic diversity shows strong variation along geographic and climatic gradients – especially latitudinal and altitudinal – for traits such as body size and ventral coloration. In contrast, genomic divergence remains the strongest predictor of multivariate traits like overall morphology. These findings underscore the value of integrating species diversity within a phylogeographic framework to better understand their evolution and the origins of phenotypic variation across spatial and temporal scales.

CONSERVATION

Novel eDNA metabarcoding applications accelerate species rediscoveries, conservation assessments, and on-site community characterization in amphibian diversity hotspots

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Abstract

In the face of steep continued biodiversity decline, improved access to field-deployable, cost-efficient, non-invasive, and scalable molecular species detection tools is of primary importance. This is especially critical around tropical ecosystems - the very areas that harbour the highest levels of biodiversity but often lack sufficient resources for comprehensive research. We highlight several of our recent methodological advancements and real-life applications in using environmental DNA (eDNA) metabarcoding to aid species rediscoveries, conservation assessments, and community characterization in remote and understudied amphibian assemblages. First, we used eDNA metabarcoding to detect threatened, elusive, and lost amphibian species across Andean Ecuador. Besides leading to the rediscovery of species not encountered by visual surveys in several decades, metabarcoding 'bycatch' revealed the presence of flagship species potentially aiding habitat conservation, but also local threats such as pathogens, invasive species, and agricultural encroachment, highlighting how eDNA data can simultaneously inform broader species conservation assessments. Second, we introduce a novel, field-deployable workflow based on isothermal Recombinase Polymerase Amplification and Nanopore sequencing that combines community metabarcoding with the on-site creation of a site-specific ('de novo') reference database directly informed by non-invasive single-species barcoding. Applied to an understudied amphibian community of high conservation relevance in northern Colombia, we show how this approach strongly improved OTU assignment while enabling a characterization of community composition. Finally, we discuss how these ongoing efforts to improve field-based community metabarcoding can help to further democratize biodiversity science in resource-limited regions, and bridge critical

knowledge gaps around the distribution and diversity of imperilled amphibian communities.

BIOGEOGRAPHY

Genomic phylogeography of a leaf-litter frog reflects ecoregional partitioning of Central African rainforests and reveals partial but notable mitonuclear discordance

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Abstract

Selecting an appropriate model species within a phylogeographic framework can provide insight into the history of the ecosystem that the species inhabits. In this study, we investigate genomic and mitochondrial DNA polymorphisms in a leaf-litter frog from lowland rainforests of Central Africa, *Phrynobatrachus auritus* (Phrynobatrachidae). Genomic polymorphisms support five to six population units that correspond well to five ecoregions of lowland/coastal forests. Two gene pools (evolutionary units) are found in the Central Congolian Lowland, one in the Northeast Congolian Lowland, one split into two subunits in the Congolian Coastal and Northwest Congolian Lowland, and one in the Cross-Sanaga-Bioko Coastal Forests ecoregions. The deepest phylogenetic split separates the evolutionary lineages south and north of the Congo River, highlighting the importance of the Central Congolian Lowland Forests for biodiversity. The nuclear genomic polymorphisms partially support the mitochondrial structure, but considerable mito-nuclear discordance has also been found in present-day southern Gabon. One deeply divergent mitochondrial lineage is part of geographically larger nuclear gene pool (panmictic population) containing also several other mitochondrial lineages. Nuclear genomic admixture likely occurred upon secondary contact of previously fragmented populations expanding from Pleistocene forest refugia, while maternal philopatry has likely caused the maintenance of geographically restricted divergent mitochondrial lineages.

CONSERVATION

Genomes on a Ferry: Human-Mediated Translocation and Hybridization in *Cryptoblepharus* skinks

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Abstract

In 2020, unidentified *Cryptoblepharus* skinks were first observed on Tromelin Island in the Western Indian Ocean—an uninhabited island lacking any natural terrestrial reptile fauna. To uncover the biogeographic origin of these lizards, targeted sampling and whole-genome sequencing were undertaken, resulting in a wider phylogeographic project. The novel population was identified as a hybrid of two species from separate islands, each located over 1000 km from Tromelin. Nuclear genomes revealed a chromosomal mosaic of the parental species. This indicates recent secondary-contact hybridization, as confirmed by multiple population genomic approaches including admixture analyses, f-branch statistics, FST, and HybridDetection. Coalescent modeling linked the introduction of the skinks to ferry routes between 2011 and 2019—the main anthropogenic vector between the islands. This event led to demographic bottlenecks in the founder population and a female-bias from one parental species. This study provides clear genomic evidence of anthropogenic translocation and hybridization in an island reptile, underscoring both evolutionary consequences and biosecurity risks. It represents one of the few documented cases in which anthropogenic vectors for the introduction of reptile species have been directly identified through genome-wide molecular evidence and offers a unique window into hybridization dynamics unfolding. I will guide the audience through genomic detective work, methodological thought processes, and the procedures involved in investigating inadvertent human-mediated introductions in island reptile populations using modern scientific tools.

ECOLOGY

The Feasibility of Faeces: Advancing Non-invasive Frog Dietary Ecology with DNA Metabarcoding

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Abstract

Tropical amphibians play key roles in ecological networks, yet their diets remain poorly characterised. Traditional methods such as stomach flushing or dissection are often invasive or even fatal, limiting their suitability for conservation research. This project aims to develop and validate a non-invasive DNA metabarcoding workflow for amphibian dietary analysis, enabling ethical and scalable ecological study. A total of 104 faecal samples were collected from captive frogs at Manchester Museum and are now being processed to test the effectiveness of different preservation and extraction methods. This includes assessing whether dietary profiles detected in faeces reflect known feeding regimes – in other words, does what goes in come out? Preliminary results suggest that the PowerFecal Pro kit yields higher DNA quantities than standard approaches, while ethanol performs consistently and remains cost-effective and widely available. These trials also address challenges such as DNA degradation and PCR inhibition. Following sequencing, dietary DNA will be translated into ecological information through taxonomic assignment and comparative analysis. In 2026, the workflow will be field-tested in Amazonian Peru using faecal samples from the same species and sites as 124 archived gut content samples. This will enable direct comparison of dietary profiles and assess whether non-invasive methods can generate data suitable for species monitoring and trophic analysis. By integrating method development with ecological application, this work contributes practical tools for amphibian research and supports more ethical study design through adherence to the 3Rs (Replacement, Reduction, and Refinement).

TAXONOMY

Uncovering Phylogenetic Relationships of *Anodonthyla* Frogs (Microhylidae: Cophylinae) and Resolving the *A. boulengerii* Species Complex

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Abstract

The recent completion of the DNA barcode inventory of Madagascan frogs revealed a substantial gap in the recognized anuran diversity through the identification of many formerly unknown species that are still awaiting taxonomic description. This included six species for the genus *Anodonthyla* Müller, 1892, a group of mostly arboreal cophyline frogs (Anura: Microhylidae: Cophylinae) that are endemic to Madagascar. Three of these species have been described over the past years, bringing the number of currently recognized taxa for the genus to 12 species. However, taxonomic uncertainties continue to exist within the genus, particularly with respect to *Anodonthyla boulengerii* Müller, 1892. This species was described with the imprecise locality 'Madagascar' and individuals assigned to the species form a species complex comprising several lineages of uncertain taxonomic affiliation. Due to the almost

complete absence of morphological differentiation, these cryptic species have formerly been lumped together under a single taxonomic name.

The project's objective was to do a molecular revision of the genus *Anodonthyla*, focusing on the *Anodonthyla boulengerii* species complex. For this purpose, publicly available sequences were complemented with newly generated DNA sequences for four mitochondrial (12S, 16S3, 16S5, COI) and three nuclear markers (BDNF, CMYC, Rag1). The revised multi-gene phylogeny and several methods for species delimitation (e.g. ABGD, ASAP, PTP) showed that the *A. boulengerii* species complex is not monophyletic but comprises seven distinct lineages. In addition, two new candidate species outside this species complex could be identified. This study represents a first attempt to re-assess the species diversity of *Anodonthyla* frogs and unravel the *A. boulengerii* species complex to facilitate the description of remaining candidate species for the genus.

EVOLUTION

Genomic insights into the population structure of India's "Big Four" medically important snake species

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Abstract

Snakebite envenomation is a neglected tropical disease affecting over 2.5 million people globally each year. The WHO estimates 81,000 to 138,000 deaths annually, with India bearing a major burden—about 58,000 deaths and 140,000 permanent disabilities. Four venomous species—*Naja naja* (Spectacled Cobra), *Bungarus caeruleus* (Common Indian Krait), *Daboia russelii* (Russell's Viper), and *Echis carinatus* (Indian Saw-scaled Viper)—cause most of these fatalities and are collectively termed the “Big Four.” Antivenom in India is produced using venom from these species, yet regional variation in venom composition and antivenom efficacy has been widely reported. While prior studies have explored venom biochemistry and clinical effects, the population structure of these species remains poorly understood. We use whole-genome sequencing on samples collected across India to assess the genetic structure in all four species. This is the first comparative genomic study of the Big Four, offering insights into intraspecific divergence, gene flow, and potential drivers of venom variation.

ECOLOGY

More than the sum of its parts: transcriptomic insights into gene expression responses to interacting environmental stressors

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Abstract

Developmental plasticity can provide an adaptive advantage in heterogeneous environments. However, organisms are generally confronted with multiple stressors at a time, and how responses to simultaneous stimuli are regulated is not always clear. This is particularly interesting when environmental stressors act on the same traits, but invoke a response in different directions. The development of some amphibian tadpoles is particularly sensitive to pond desiccation, which can trigger faster development and earlier metamorphosis. Predator presence may result in largely the opposite reaction to pond desiccation, slowing development and investing instead in growth. Despite both responses being coordinated by the HPI axis, it is not clear whether these stimuli are truly conflictive and how physiological and morphological responses are governed at the molecular level. To understand what molecular mechanisms are driving such complex changes, we therefore use a transcriptomic approach to characterise the network of environmentally sensitive genes in *Pelobates cultripes* tadpoles that are activated in the face of pond drying, and predation risk, and the combined stimuli. We use a modelling framework to test whether gene expression under combined threats is additive, antagonistic or synergistic in nature. Interestingly, we find that across different tissues, gene expression responses to the two pure stimuli are largely independent from each other, with genes that are differentially expressed in both being correlated in the direction of their response. Moreover, the responses to combined stressors are much larger than to either stressor on their own.

ECOLOGY

Species distribution models predict genome-wide polymorphism and gene flow in the Yellow-bellied toad (*Bombina variegata*)

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Abstract

Over the past two decades, the rise of citizen science has greatly expanded the availability of species presence data, supporting the use of increasingly sophisticated species distribution models (SDMs). While SDMs are widely used to infer relative habitat suitability (RHS) for biodiversity monitoring and conservation planning, the extent to which RHS captures population-level demographic and genetic processes remains unclear. Here, we examined the relationship between SDM-predicted habitat suitability and both genetic diversity and gene flow in the Yellow-bellied toad (*Bombina variegata*), at the southeastern edge of its distribution.

We sampled 404 individuals across 92 populations within a ~70,000 km² area and generated 37,169 high-quality SNPs using RAD sequencing. In parallel, we compiled ~10,000 high-quality presence records to develop two hierarchical SDMs including a bioclimatic model at the lineage range level and a land-use model at a fine regional level. Using a multi-scale modelling framework, we assessed how well each RHS metric explains spatial patterns of genetic diversity and connectivity. We also examined how the contribution of individual variables varies across spatial scales. To model gene flow, we used gravity models that integrate population size, to account for the differential influence of genetic drift among populations.

Our results show that RHS derived from hierarchical SDMs predict both genetic diversity and gene flow in *B. variegata*. However, the predictive power of RHS for genetic diversity is strongly scale-dependent, highlighting the importance of multi-scale approaches when using SDMs to infer genetic patterns in amphibians. Additionally, gravity models outperformed traditional pairwise-distance approaches, highlighting the importance of incorporating population-specific drift into landscape genetic analyses.

OTHER

Amazonian leaf frogs' skin chemistry: metabolite variation among Phyllomedusidae species

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Abstract

Bioactive peptides have been sought after for decades in the epidermal secretions of South American anurans, and several have been identified, isolated, and characterized. The first bioactive peptide isolated was from a species of the genus *Phyllomedusa*, currently considered a rich source of biologically active peptides with antimicrobial, neuromodulatory, and pharmacological potential. Despite the increasing biomedical interest, comparative data on the chemical composition of Amazonian species remain scarce. Here, we present an exploratory metabolomic characterization of the venom

from six Phyllomedusidae species collected across the Brazilian Amazon region, aiming to map low-molecular-weight compounds that complement the peptide repertoire of these frogs. Skin secretions of *Phyllomedusa bicolor*, *Phyllomedusa tarsius*, *Phyllomedusa camba*, *Callimedusa tomodopterna*, *Pithecopus palliatus*, and *Pithecopus hypochondrialis* were analyzed by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and processed using an annotation pipeline combining database searches with manual curation. A total of 70 compounds were annotated and distributed among key chemical classes. The skin poison's dominant fraction consisted of free amino acids and their derivatives, comprising 48-57% of the total signal of each species. Alkaloids had the second highest abundance, ranging from 7-12% of total signal, except for *P. palliatus* and *P. bicolor*, which had peptides as more abundant. Peptides were mainly abundant in *C. tomodopterna*, *P. bicolor*, and *P. palliatus*, representing at least 10% of their total signal. Lipids were present in the poison of all species, accounting for 4-5% of the total. These findings expand the chemical characterization of Amazonian *Phyllomedusa* species and provide promising targets for functional studies, bioprospecting, and comparative approaches in chemical ecology and the evolution of toxins among anurans.

CONSERVATION

Isolated adder (*Vipera berus*) populations: utilising low-medium coverage genomics to identify and assess the effects of barriers on gene flow across fragmented landscapes

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Abstract

Globally, reptile populations are under continuous pressure from human encroachment, leading to increasing habitat fragmentation, isolation of populations and population declines. The result is scattered, small pockets of suitable habitat which harbour genetically isolated populations at increased risk of inbreeding, reduced fitness and ultimately, localised extinctions.

Adder (*Vipera berus*) populations in the UK and across Europe consist of small genetically isolated populations, once part of larger populations which have become fragmented by human activities and structures such as roads, agricultural land, and cities.

Utilising low to medium coverage genomics we identify barriers to gene flow between adder populations, both natural (e.g., marine straits) and man-made. We also assess the genetic health, diversity, and structure of genetically isolated adder populations. We highlight the genomic consequences of inbreeding by identifying homozygotic runs, often going unnoticed in populations as they progress towards or have recently experienced genetic bottlenecks. Our results will better inform future conservation efforts and guide methods from a genomic perspective not only for adders in the UK, but reptile populations globally as human encroachment continues.

SYSTEMATICS

Systematics of Sphenomorphin Skinks in Western Melanesia: A Phylogenomic Approach

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Abstract

Western Melanesia, including New Guinea, sits at the crossroads of Asia, Australia, and the Pacific Islands and is one of the most interesting, yet understudied, hyper-diverse regions on Earth. The region's dynamic geological history has led to extreme topographic heterogeneity and complex evolutionary histories for its biota. Despite extremely high diversity and endemism, the origins of most taxonomic groups in the region remain poorly studied. This is especially true for range-restricted taxa such as reptiles. Represented by 11 genera in Western Melanesia, sphenomorphin skinks represent a significant proportion of the region's lizard diversity. Despite high species diversity and many recently described species, the evolutionary relationships within this group remain poorly understood. In this study, we address this knowledge gap by employing advanced high-throughput sequencing methods to generate genetic data for over 40 sphenomorphin skinks from across Western Melanesia. DNA was extracted using established protocols from fresh and historical samples, and novel squamate conserved loci (SqCL) sequence data was generated. Our preliminary results highlight the complexity in this highly diverse group, and the paraphyly of multiple genera.

SYSTEMATICS

Past and Present of Herp-Omics: From Sanger Sequencing to Museomics

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Abstract

Over the past three decades, biodiversity assessments have undergone a major shift in methodology and scope, driven by rapid progress in techniques to generate molecular data. From the early beginnings of relying on Sanger sequencing to receive fragments of mitochondrial and nuclear loci, to the present era that is dominated by genome-scale data from high-throughput techniques, such as shotgun sequencing, target capture, transcriptomics, and museomics – the nature of questions that we can address with sequencing techniques is broader than ever.

Before the advent of -omics techniques, herpetological research was limited by reliance on mostly morphological characters, in which plesiomorphy and plasticity often led to misidentification of species or life stages, misled phylogenetic reconstruction, and overlooked cases of hybridization. While the molecular revolution has dramatically enhanced practice in species delimitation, systematics, and drawing evolutionary relationships, notable challenges remain – particularly the exceptionally large and complex genomes of many amphibians, limited genomic resources for non-model taxa, and difficulty achieving sufficient sampling coverage in remote or understudied areas. International collaborative efforts and consortia are now starting to address these long-standing challenges more holistically. Together, the molecular revolution and the shift toward broader collaboration are shaping a new era in herp-omics, helping to address long-standing challenges, enhance biodiversity assessments, and foster more consistent, and cooperative research across the field.

CONSERVATION

Genetic LIFElines for the Aeolian Wall Lizard: The Role of Conservation Genomics in the EOLIZARD Project

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Abstract

Podarcis raffonei, the Aeolian wall lizard, is among Europe's most endangered reptiles, with only four isolated populations surviving on remote volcanic islets and a peninsula. Within the LIFE EOLIZARD project (LIFE22-NAT-IT-LIFE EOLIZARD, Project 101114121), we are applying conservation genomics to support targeted interventions, including the removal of the invasive congener *P. siculus* from the islets of Lisca Bianca and Bottaro, and the establishment of a Sanctuary of *P. raffonei* in their place. To guide the *P. siculus* translocation, we first performed a genetic analysis of individuals from Lisca Bianca, Bottaro, and Panarea. The results confirmed a shared

genetic pool between Lisca Bianca and Bottaro with Panarea, validating the relocation of individuals to this nearby island. In parallel, we conducted a genome-wide screening of over 200 *P. raffonei* individuals from Scoglio Faraglione and Capo Grosso to select up to 40 breeding pairs per site for ex situ conservation. Selection was based on three key criteria: 1) maximizing intra-group genetic diversity; 2) minimizing relatedness to avoid inbreeding; and 3) excluding hybrids with *P. siculus*, particularly from Capo Grosso, where introgression has been detected. These genomic insights have been critical in shaping conservation decisions and ensuring the long-term viability of the species. LIFE EOLIZARD exemplifies how integrative genomics can drive effective, science-based recovery plans for highly threatened island endemics.

EVOLUTION

Towards complete genomes of non-model vertebrates

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Abstract

Recent technological developments have finally made sequencing even the largest amphibian and reptilian genomes feasible. However, when aiming at understanding the genomic basis of differences between populations or species, the most variable regions of genomes such as highly repetitive centromeres, sex chromosomes, population-specific B chromosomes, and germline-restricted chromosomes remain difficult to study in many non-model vertebrates. Here I discuss ongoing projects aiming at characterizing the complete landscape of genetic diversity in non-model vertebrates.

EVOLUTION

Unleashing The Secrets Of Iberian Snakes: Exploring Their Venom Evolution Through Multi-Omics

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Abstract

Venom is a complex and fascinating evolutionary trait that has independently emerged multiple times across the animal kingdom. In snakes, venom is synthesized in specialized glands – venom glands – and serves primarily for predation or self-defensive purposes, reflecting intense ecological pressures and offering a unique window into evolutionary innovation. Despite advances in molecular systematics, many phylogenetic relationships –particularly among colubroid snakes – remain

ambiguous or contested, limiting our understanding of venom evolution in its broader genomic context.

The overall aim of the project is to investigate the evolution and diversity of venom in Iberian snakes through an integrative multi-omics approach. Specifically, we are generating and analysing high-quality reference genomes, venom-gland transcriptomes, and proteomic profiles for all snake genera native to the Iberian Peninsula. This effort will allow us to compile comprehensive catalogues of venom-gland-specific toxin genes—the so-called venom-ome—representing the functional core effector proteins of envenomation.

Simultaneously, population genomic analyses will be carried out using whole-genome resequencing data to explore phylogenetic relationships, demographic history, inbreeding, and population structure across species and subspecies. These analyses will help resolve long-standing taxonomic uncertainties and provide new insight into the evolutionary processes shaping snake diversity in the region.

As a proof of concept, we present the assembly of five high-quality genomes and preliminary results from the population genomics of *Malpolon monspessulanus*. Ultimately, this project will produce valuable genomic resources, refine the phylogenetic framework of Iberian snakes, and deepen our understanding of venom evolution—contributing to both biodiversity conservation and potential biomedical applications.

EVOLUTION

Chromosome-scale haplotype-resolved reference genomes of four species of the Iguanidae reveal genomic basis of adaptations of the Galápagos marine iguana (*Amblyrhynchus cristatus*)

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Abstract

After the colonization of the Galápagos archipelago by their common ancestor approximately eight million years ago, the Galápagos iguanas diverged into two ecologically highly distinct lineages – the terrestrial *Conolophus* genus, and the Galápagos marine iguana (*Amblyrhynchus cristatus*), which adapted to the marine environment, by transitioning to foraging on macroalgae in the intertidal and subtidal zones. Therefore, the Galápagos iguanas, together with other closely related iguanids, represent a compelling system to study both the genomic effects of colonization of remote island systems and the genomic basis of adaptations to the marine environment. Here we present the chromosome-scale, haplotype-resolved assemblies and genome annotations of four iguanid species: the Galápagos marine iguana (*Amblyrhynchus cristatus*), Galápagos land iguana, *Conolophus subcristatus* and two species from the two most closely-related mainland lineages, *Cachryx defensor* and *Ctenosaura palearis*. Their genomes show high chromosomal-synteny and stability in genome size and repeat content. We analyzed gene family size-changes and conducted scans for positive selection and shifts in selection intensity to detect candidate genes associated with adaptation to both the Galápagos ecosystem and the marine environment within this system. Our preliminary results suggest that gene families of diverse detoxification proteins have contracted in the marine iguana, potentially linked to its dietary shift from terrestrial plants to marine macroalgae.

BIOGEOGRAPHY

Systematics and biogeography of the New Guinean crown snakes (*Aspidomorphus*: Elapidae)

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Abstract

The New Guinean crown snakes, genus *Aspidomorphus*, are poorly studied. They occur across the major biogeographic regions of New Guinea: Accreted Terranes (AT), Vogelkop Composite Terrane, East Papuan Composite Terrane (EPCT), Maluku, the Bismark/Admiralty Islands, and Australian Craton, but they have few records in the Central Cordillera. Of the four named species, *A. dimorphus* is endemic to Sudest Island, in the EPCT, *A. schlegelii* is restricted to the north-central Accreted Terranes and Vogelkop regions, *A. lineaticollis* is restricted to the EPCT and parts of the AT, and *A. muelleri* is widespread from Maluku in the west to New Ireland in the east. A previous study using Sanger sequencing data identified seven deeply divergent lineages within *Aspidomorphus*, although sampling was mostly focussed on eastern New Guinea. In our study, we increased geographic sampling and sequenced thousands of loci. We present a fossil-calibrated phylogeny and historical biogeographic analyses for *Aspidomorphus*, using data from additional Sanger sequencing and from ultra conserved elements (UCE) from fresh and historic museum samples distributed across New Ireland, New Guinea and Maluku. Our results reveal complex evolutionary relationships between lineages on the EPCT, Accreted Terranes, Vogelkop, Bismarck/Admiralty and Australian Craton. The genus first started to speciate within the Oligocene, with most major divergences occurring throughout the Oligocene and Miocene. These events would have been prior to the formation of modern New Guinea, and therefore lineages would have been evolving on a series of proto-New Guinean islands.

EVOLUTION

Introgression landscape in Australian burrowing frogs *Neobatrachus*

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Abstract

Whole genome duplication (WGD) provides both evolutionary opportunities and challenges, particularly for animal lineages where successful polyploid establishment is rare. The Australian burrowing frog genus *Neobatrachus* presents a unique system, containing six diploid and three independently derived tetraploid species (*N. sudellae*, *N. aquilonius*, and *N. kunapalari*). Using population genomic analyses, we reveal that patterns of admixture and introgression across the genome are highly heterogeneous among *Neobatrachus* species. Notably, in *N. kunapalari*, chromosome-specific signals—especially on Chr1 and Chr2—strongly deviate from the genome-wide ancestry profile, suggesting a complex hybrid origin involving at least two distinct hybridization events. Mitochondrial phylogenies and ancestry deconvolution analyses further support the hypothesis that *N. sutor* contributed genetic material as either a maternal or paternal donor in these events. Our study also uncovers evidence of ongoing gene flow, including the formation of recent hybrids between diploid species pairs such as *N. fulvus* × *N. sutor* and *N. pelobatoides* × *N. albipes*. Together, these findings provide a detailed landscape of introgression in *Neobatrachus* and highlight the roles of recurrent hybridization and WGD in shaping the evolutionary trajectories and sex chromosome dynamics of polyploid vertebrates.

Symposium

Multifaceted evolution of amphibian and reptile phenotypes in changing environments

Organisers: Giulia Simbula, Lekshmi B. Sreelatha, Nahla Lucchini, Prem Aguilar & Miguel A. Carretero



ECOLOGY

Balancing visibility and concealment: drivers of dorsal colour pattern variation in wall lizards

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Abstract

Colouration and its perception play a fundamental role in survival by mediating key biological functions such as camouflage and mate recognition in many organisms. These traits are often subject to multiple, and sometimes opposing, selective pressures giving rise to phenotypic diversity. In this study, we test alternative hypotheses concerning the persistence of dorsal colour pattern variation in *Podarcis lusitanicus* (Lusitanian wall lizard) populations from NW Iberian Peninsula. We conduct

a quantitative assessment of colour-pattern variation using Quantitative Colour Pattern Analysis (QCPA) on 470 individuals from 21 populations. We model the lizard appearance for the spectral sensitivities of a conspecific and an avian predator for different distances. First, we test whether colour-pattern metrics correlate with body size or differ between sexes, and whether conspecifics or predators can discriminate between male and female colour patterns at different distances. We then calculate discriminability indices (Just Noticeable Differences, JNDs) between the lizards and their habitat backgrounds to test whether dorsal colouration facilitates camouflage. Specifically, we assess whether individuals are better matched to their local habitat backgrounds when viewed by avian predators. Finally, we assess how environmental variables (e.g., local climatic variables) influence discriminability to explore the extent to which local ecological conditions shape colour-pattern effectiveness. Together, results provide insights into the ecological and evolutionary drivers maintaining colour pattern diversity in *P. lusitanicus*, contributing to a broader understanding of how visual signalling and camouflage strategies evolve under complex selective landscapes.

ECOLOGY

Herpetology in new and ancient cities

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Abstract

Urbanization presents a powerful and rapidly expanding force of environmental change, shaping ecosystems through altered landscapes, microclimates, and resource distributions. Understanding how organisms adapt to these anthropogenic pressures, both past and present, is critical to predicting ecological resilience and evolutionary trajectories. Wall Lizards (genus *Podarcis*) and Fence Lizards (genus *Sceloporus*) are widely distributed, abundant, ecological generalists that thrive in a variety of ecosystems, including cities. I will present research on morphological, color, and ecophysiological shifts in response to urbanization in new (Los Angeles and San Diego) and ancient Mediterranean cities (Taormina, Sicily and Cycladic islands, Greece). My students and I have found similar and repeated shifts in response to urbanization in lacertids and phrynosomatids from new cities around the world. Additionally, we have found microenvironmental differences between new cities, ancient cities, and non-urban areas that have implications for ectotherm ecology, persistence, and adaptation to these novel human-made ecosystems. Ultimately, this research seeks to foster a deeper understanding of how trait-environment interactions govern ecological success or vulnerability in herpetofauna facing human mediated climate change and land-use shifts. My lab aims to identify general principles governing phenotypic evolution in human-altered landscapes, offering a blueprint for predicting species responses to the Anthropocene and informing conservation strategies.

ECOLOGY

Do Mediterranean reptiles occupy a specific functional niche? A first approach with lacertids

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Abstract

Understanding the functional and evolutionary responses of organisms to environmental variation is relevant for research and conservation. Being ectotherms and sedentary, suffering considerable environmental changes, playing a pivotal ecosystem role and providing substantial ecosystem services, lacertid lizards exemplify the conflict between conservation and development. Recent research provides 1) a robust phylogenetic hypothesis, paleoecological timeframe and biogeographic framework; and 2) physiological parameters for functional inference on thermal, hydric and trophic ecology, available for multiple species. As such, the groundworks are set to investigate whether Mediterranean and non-Mediterranean species differ functionally and derive conservation consequences. Lacertid phylogeny display early cladogenesis (60 Ma) and diversification of the dominant clade in Europe, the Lacertini (35 Ma). Mediterranean Basin is geologically dynamic with multiple instances of vicariance and dispersal. However, paleoclimatology suggests that Mediterranean climate, defined by summer drought, is relatively young (3.4 Ma). Thus, the main lacertid lineages did not evolve under the current climate, but responded to climate shifts with either adaptation, range decline or extinction. Preferred temperatures are essentially conservative, both in the phylogeny and across populations, while water loss rates are more flexible. Although most species are generalist invertebrate eaters, gigantism and predator release repeatedly promoted trophic niche enlargement. Remarkably, thermal, hydric and trophic traits trade-off: dehydration increases with temperature; hydoregulation restricts thermoregulation when water is limited; and herbivory is associated with high preferred temperatures. Species trade-offs, climate and other environmental constraints can be represented in a 3D space to answer the initial question, illustrated here with Iberian lacertid data.

MORPHOLOGY

Turning a turtle into a box: The evo-devo of shell kinesis

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Abstract

Turtles arguably feature a low number of potentially variable (evolvable) character states and are therefore more likely to convergently evolve similar phenotypes. In this talk, I address anatomical alterations related to the convergent evolution of turtle shell kinesis, which has arisen independently at least eight times. Shell kinesis describes active movement of shell bones via articulations or “hinges”, which arise via similar skeletal remodeling processes in distantly related “box turtles”. I describe complex diarthrodial joints and modified muscle connections that expand the functional boundaries of the limb girdles and neck in different turtle lineages. I show that depending on evolutionary history and structural correlations, a plethora of anatomical alterations and developmental changes has led to similar functional outcomes during the convergent evolution of turtle shell kinesis. Further, evolved allometric shifts in skeletal dimensions are crucial to the delayed maturation of this complex trait. The various iterations of this intricate phenotype exemplify the potential for the turtle musculoskeletal system to undergo evolutionary change, despite developmental constraints imposed by the structural intricacy of a shelled body plan.

ECOLOGY

Modelling competition under current and future climate change scenarios in a pair of competing lacertid lizards

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Abstract

Understanding how interspecific competition shapes species responses to climate change remains a critical gap in future species distributions and population viability. While mechanistic niche models have advanced environmental forecasting, most approaches do not tackle biotic interactions. This study presents the first steps toward a framework for mechanistically modeling thermal interference competition in ectothermic vertebrates. We developed dynamic energy budget (DEB) models integrated with NicheMapR microclimate and biophysical modeling for two competing lacertid lizards, *Iberolacerta horvathi* and *Podarcis muralis*, across 13 syntopic sites spanning across and elevational gradient. Experimental studies show *P. muralis* competitively displaces *I. horvathi* from optimal basking sites, creating asymmetric thermal resource access crucial for ectotherm performance. Our mechanistic framework explicitly incorporates thermal interference by forcing the subordinate species into suboptimal conditions (30%, 50%, or 80% shade) when the dominant species occupies prime basking locations. Competition scenarios were implemented by determining *P. muralis* activity patterns, then creating shade constraints for *I. horvathi* simulations. This captures realistic asymmetric competition where interference competition dictates thermal resource access. Our results reveal the effects of thermal interference on subordinate species life history traits, with increasing competition delaying reproductive maturity and reducing lifetime reproductive output. Here we present results comparing time to first reproduction and net intrinsic rate of population increase under 3 different competition scenarios and under current as well as future potential climate scenarios. The framework establishes essential tools for forecasting species interaction outcomes under environmental change, with broad applications for ectotherm thermal and community ecology and conservation biology.

EVOLUTION

Convergence and ancestral regulatory variation upstream of BCO2 explain the maintenance of colour morphs across the radiation of wall lizards

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Abstract

In the common wall lizard (*Podarcis muralis*), ventral carotenoid coloration is determined by a regulatory region upstream of the beta-carotene oxygenase 2 (BCO2) gene. However, previous evidence is not clear on whether this regulatory element explains the similar phenotypes found across highly divergent lineages of the same species, as well as other *Podarcis* species. To test whether convergent evolution of BCO2 explains differences in carotenoid coloration, we conducted targeted sequencing of a 250 kb region around BCO2 in yellow and white individuals from two lineages of *P. muralis* that diverged approximately 2 million years ago. Our results indicate that a 200 bp region, ~3.7 kb upstream of BCO2, explains differential carotenoid deposition in both lineages. However, we did not detect any evidence of selective sweeps across this region, indicating that white and yellow haplotypes might be under long-term balancing selection. To test this idea, we generated pool-seq data from 11 *Podarcis* species which also exhibit both colour phenotypes and screened their genomes for regions showing marked differences in allelic frequencies. The results indicated that the same region upstream of BCO2 differed between white and yellow morphs in seven species, whereas in three of them we did not identify such a pattern. Interestingly, we detected a deletion of ~1kb overlapping the causal region in the yellow morph of *P. tiliguerta*. Overall, our results suggest that a combination of balancing selection, convergent evolution and perhaps introgression explain the evolution of this widespread polymorphism in wall lizards. Given the cis-regulatory nature of the underlying mutations, we setup to test if it carries any effect in neighbouring genes, namely the equally distant interleukin-18 (IL18). Using RNA-seq data, we found that the expression of BCO2 and IL18 was positively correlated, possibly explaining the link between colour and immunity previously reported in *Podarcis* lizards.

ETHOLOGY

The relevance of visual ecology on the design of lizard social visual signals

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Abstract

Animal colour diversity reflects a complex interplay of evolutionary processes, especially in traits involved in visual signalling. While conspicuous patches enhance signal efficacy, they may also increase the risk of detection by predators. Conspicuous colour signals are common in vertebrates, including many lizard clades. Lacertid lizards often display conspicuous lateral or ventrolateral patches presumably involved in male-male competition. These patches typically reflect in the ultraviolet (UV)-blue range, increasing conspicuousness within the lizard's visual spectrum. Yet, some species exhibit UV-green or UV-yellow patches, or lack such conspicuous patches. As lacertids occupy a wide range of habitats with contrasting visual environments, we hypothesised that the balance between conspicuousness and predator detection shapes signal design. Specifically, we expected phenotypes to differ in conspicuousness (UV-blue > UV-green > UV-yellow > no-patch), with more conspicuous ones occurring in visually complex habitats. To test this, we adopt objective colour measurement techniques (spectrophotometry, visual modelling, UV photography) and a comparative phylogenetic analysis. First, we tested whether differences in conspicuousness match microhabitat segregation in three representative species: *Psammodromus algirus* (UV-blue), *Psammodromus edwardsianus* (UV-green), and *Acanthodactylus erythrurus* (UV-yellow), which may occur in sympatry but use distinct microhabitats. Second, using a new comparative method for ancestral reconstruction of multi-state categorical traits, we tested

whether conspicuousness is related to environmental complexity across 343 species. Our results confirm differences in conspicuousness among patch types and species-specific microhabitat use. The comparative analysis shows more conspicuous patches in species from visually complex habitats. Altogether, our findings support that visual ecology constrains colour signal diversity in lacertids.

MORPHOLOGY

Elevational patterns of morphological evolution in Harlequin Toads

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DGHT Grantee

Abstract

The effect of environmental variables on the morphology of animals show taxon-specific patterns, with varying levels of consistency across lineages. In endotherms, body size often correlates with latitude or elevation, but in ectotherms such as amphibians, the pattern is less consistent. The genus *Atelopus* (Anura: Bufonidae) exhibits remarkable variation across its range, offering an ideal group to investigate how environmental gradients drive morphological evolution. This genus is distributed across a wide range of elevations in the Neotropics, from lowland rainforests to high Andean mountains, providing a natural experiment to explore trait-environment relationships. We conducted a phylogenetically informed comparative analysis, assuming the most recent published phylogeny of the genus, to evaluate how elevation and associated climatic variables relate to the morphological evolution of *Atelopus*, focusing on correlations among body size, robustness, and limb proportions. Morphological measurements were obtained from museum specimens, encompassing over 70 named and unnamed species. Our results showed a clear phylogenetic signal of body size and shape, and higher-elevation populations exhibiting larger body sizes and relatively shorter limbs. Elevation emerges as a predictor of morphological evolution in *Atelopus*, offering new insights into the adaptive history of the genus. Our findings contribute to a broader understanding of how environmental factors shape morphological diversity in ectotherms and highlight the importance of integrating evolutionary and ecological perspectives in biodiversity research. Identifying these trait-environment relationships may also help reveal patterns associated with species vulnerability, offering additional insights that can inform conservation strategies for threatened taxa like *Atelopus*.

EVOLUTION

Colour polymorphisms in Podarcis lizards, what a problem!

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Abstract

Colour polymorphisms are of great interest in evolutionary biology, and studies on lizards have provided valuable clues to understand how evolution promotes and maintains them. The ventral colour polymorphism of many *Podarcis* species has attracted much attention, providing some outstanding advances in the general study of colour polymorphisms, but being far from conclusive interpretations on its evolutionary meaning. Given the different approaches employed to study coloration, questions such as the number of morphs, whether mosaic phenotypes constitute true morphs, or whether alternative reproductive strategies underlie the maintenance of the polymorphism remain an open debate. This hinders progress in the field, but at the same time generates multiple challenging hypotheses. Here, I review what we know about ventral colour polymorphisms in *Podarcis*, describing its parameters, identifying what should be considered a morph, and discussing new available evidence that agrees or refutes the central hypotheses considered to date. *Podarcis* lizards can show up to four ventral colours: UV-reflecting white, non-UV-reflecting white, yellow and orange. These colours can be either expressed as pure morphs, or as mosaics when orange does not evenly cover the scales of a lizard (e.g. yellow-orange). This colour variation meets the premises of a polymorphism because they are categorically discriminable, have a genetic basis, and are stable at maturity. However, despite the many studies testing for differences between morphs (e.g. in immunology, behaviour), no clear pattern has emerged across lineages and/or species. Moreover, specific experiments testing for alternative reproductive strategies linked to morphs and the absence of cyclic changes in local morph composition argue against this hypothesis. Comparative research suggests that morph diversity is driven by both sexual and natural selection, but more efforts must be made to clarify the meaning of *Podarcis* polymorphisms.

MORPHOLOGY

Evolution of incipient toepads in a radiation of ecologically diverse geckos

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Abstract

Adhesive toepads have evolved repeatedly in a variety of clades thereby facilitating attachment to inclined surfaces in climbing species. Among vertebrates, geckos are the most thoroughly studied with regard to their adhesive toepads. Even so, the evolutionary origin of these complex structures is still elusive, this being partially due to the scarcity of studies on species with incipiently developed toepad morphologies. One suggested model for the presence of incipient toepads are the bent-toed geckos (genus *Cyrtodactylus*), an ecologically diverse radiation, the climbing members of which possess enlarged subdigital scales. It is known that a few such species carry spatulated setae as the microstructures on these scales. Given this, we explore the evolution of incipient toepad morphology in *Cyrtodactylus* species from different microhabitats (ecotypes) in relation to habitat use (terrestrial, arboreal, rock-dwelling and generalist) to unravel how and under what ecological circumstances adhesive toepads evolved. We measured size-corrected subdigital scale area, examined subdigital scale shape using 2D geometric morphometrics, and subdigital microstructures using scanning electron microscopy. We reconstructed the evolution of these traits and tested whether species occupying different microhabitats differed in their subdigital scale morphology. We found that both arboreal and rock-dwelling species had relatively larger subdigital scale areas compared to terrestrial and generalist species, with subdigital scales that incrementally approach the shape of typical adhesive pads. Microstructures, to the contrary, are more phylogenetically clustered than ecologically correlated.

CONSERVATION

Ecological relevance of Voluntary Thermal Maxima (VTMs) in the wall lizard *Podarcis muralis*

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Abstract

Understanding the responses of biodiversity to climate change requires precise knowledge of how animals cope with warming. Voluntary Thermal Maxima (VTM) have been recently proposed as ecologically meaningful measures of thermal niches that can be experimentally assessed in the field. VTM records the temperature at which the animal moves away from a sub-optimal temperature condition and is experimentally measured by placing an animal in a thermal chamber whose internal temperature is gradually increased and defined as the temperature at which the animal voluntarily leaves the thermal chamber. Correct interpretation of VTM rely on precise understanding of intrinsic and environmental predictors of an animal's experimental behaviour. Here, we analysed the dependency of VTM on environmental factors, namely temperature at the beginning of the experiment (T_0) and time of the year (season), and individual characteristics (body mass). We conducted field experiments on 57 *Podarcis muralis* individuals sampled between June 2024 and June 2025 and fitted Generalized Additive Models, considering T_0 , season, body mass and (initially) their interactions as predictors of measured VTM. We found that our predictors explained a substantial fraction of the data variance ($\text{adj-R}^2 = 0.418$, $P < 10^{-5}$). T_0 had a marked, positive effect on VTM (slope = $0.65\text{ }^{\circ}\text{C}_{\text{VTM}}/\text{ }^{\circ}\text{C}_{T_0}$, $P < 10^{-4}$), indicating short-term acclimation. Noticeably, for the same T_0 , lizards tolerated higher temperatures in spring than in summer and fall (diff = $3.2\text{ }^{\circ}\text{C}$, $P = 0.017$), consistent with a cumulative heat stress hypothesis, rather than long-term acclimation to warmer temperatures. Body mass, while theoretically relevant, showed no significant effect on VTM in our dataset ($P = 0.375$). Our study supports the utility of VTM as a field measure to characterise significant aspects of thermal niches in terrestrial ectotherm

vertebrates, provided that the physical and environmental variables are statistically controlled.

OTHER

AGROLIZARDS+ lizards' ecological role in agriculture environment

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Abstract

Lacertid lizards are pervasive in agricultural landscapes, where they can play a key functional role as mid-level consumers, yet they are still largely overlooked as potential biological control agents. Moreover, the extent of their exposure to agricultural pesticides, and their mechanisms for coping with these chemicals remain also poorly understood. In this study, we combined DNA metabarcoding and stable isotopes to assess the trophic role of the wall lizard *Podarcis bocagei*, and their contribution to pest consumption in northern Portugal vineyards. Additionally, we also determined whether and how agricultural practices may impact lizard fitness and, consequently, their ecosystem services. Lizards were sampled from seven vineyards sites undergoing different pesticides applications, classified according to an exposure toxicity ratio (ETR) approach. It calculates risk by comparing environmental exposure (PECsoil) with toxicity to organisms (NOAEL/LD50) for rats (no toxicological data available for reptiles). To assess environmental stress on lizard, we measured the following biomarkers: body condition, fluctuating asymmetry (femoral pores), and blood parasite load. *Agrotis* spp. (Lepidoptera) was the only vineyard pest identified. Both DNA metabarcoding and isotopic approaches showed consistent trophic patterns across populations, with generalist diets, high prey overlap, and limited niche divergence, regardless of pesticide exposure. In high-pesticide sites, developmental

instability increased and immune response to parasites declined. Although direct pest control by *P. bocagei* was limited, our findings suggest lizards remain trophically flexible, consuming a range of arthropods including potential pests. These results offer insights into reptile health and ecosystem functioning in agricultural settings and support the integration of reptiles into sustainable farming and conservation frameworks.

EVOLUTION

Triggers of sexual dynamic color change

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Abstract

Many frogs and toads are explosive breeders, where large groups of males compete for limited mating opportunities during a very short breeding period. In some species, males that engage in explosive aggregations rapidly and dynamically change their color. Male Asian Common toads (*Duttaphrynus melanostictus*), for example, change body coloration from brown to bright yellow during breeding, which only lasts for 1-2 days at the onset of monsoon rains. Male yellow color does not influence male mating success, but model experiments showed that males avoid yellow conspecifics and try to amplex brown models, suggesting that male yellow color acts as a visual signal mediating fast mate recognition. From a mechanistic perspective, catecholamine injections induce this color change in minutes. However, it remains unclear which stimuli, e.g. encounters with competitors and/or abiotic changes at the onset of monsoon, trigger catecholamine release and activate the dynamic color change. We investigated if environmental conditions and/or social interactions trigger the activation of dynamic color change in male Asian Common toads. We tested 96 reproductively active adult male *D. melanostictus* at the Vienna Zoo, exploring how social (three males) or single conditions, with or without heavy rain, or handling stress influence catecholamine levels and body coloration. Abiotic stressors triggered a rapid color change to bright yellow and a change in chroma, with and without social stimuli during male-male encounters. Our findings point to the idea that sexual selection exploits responses to pronounced environmental changes that initiate an extremely short breeding period, to facilitate effective visual communication during reproduction.

MORPHOLOGY

Experimental evidence for ketocarotenoid conversion in poison dart frogs

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Abstract

Poison dart frogs of the family Dendrobatidae show a remarkable diversity in coloration across the roughly 200 species in the family. In some species, bright warning coloration is largely due to iridophores and pterin pigments, while in others, carotenoid pigments play an important role. Although carotenoids are derived from the diet, some animals have the ability to enzymatically convert yellow dietary carotenoids into red ketocarotenoids, which till this point has mainly been documented in birds, fish, and some arthropods. Here, using controlled dietary experiments and HPLC-MS analysis of skin pigments, we demonstrate ketocarotenoid conversion in two species of poison dart frogs, *Ranitomeya sirensis* and *Oophaga pumilio*. When fed with common dietary carotenoids (beta carotene, lutein, and zeaxanthin), frogs accumulate red ketocarotenoids in their skin. In both species, carotenoid profiles of captive frogs largely resemble wild frogs, suggesting that these commonly available yellow precursor pigments are sufficient for the development of more complex, derived red phenotypes. Despite the fact that *R. sirensis* and *O. pumilio* contain many of the same red carotenoids, the underlying genetic mechanisms appear to be distinct in the two species, raising the possibility that the convergent evolution of red carotenoid coloration in dendrobatid frogs has evolved independently through diverse pathways.

BIOGEOGRAPHY

Phylogeographic Study of the Alpine Newt in Slovenia

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Abstract

The alpine newt (*Ichthyosaura alpestris*) is widely distributed throughout Europe, with several subspecies and morphotypes recognized, particularly in the Mediterranean peninsulas, largely based on external morphological characteristics. While extensive genetic studies using mitochondrial DNA (mtDNA) and nuclear DNA (nuDNA) have identified distinct evolutionary lineages, the Slovenian populations have remained underrepresented in broader phylogeographic analyses. This is despite the presence of two historically recognized morphotypes in the Julian Alps: *I. a. lacustris* and *I. a. lacusnigri*, as well as significant morphological variation observed across other Slovenian regions. To improve our understanding of *I. alpestris* diversity in Slovenia, we collected over 250 tissue samples from a wide range of localities across the country. Molecular analyses were performed using both mitochondrial markers cytochrome b (CytB) and NADH dehydrogenase subunit 2 (ND2), and a nuclear growth hormone gene (GH). The resulting data reveal high haplotype diversity, suggesting that Slovenia may have acted as a cryptic glacial refugium for *I. alpestris*. Two major mtDNA clades, Northern Balkans and Romania clade (NBR) and Eastern/Western Europe and Spain clade (EWES), were identified within Slovenian populations. In Kozjansko, Boč, and Gorjanci, most individuals belong to the NBR clade, although some EWES haplotypes were also found, indicating secondary contact. Populations from the Triglav region of the Julian Alps form two distinct subgroups within the EWES clade, also displaying high haplotype diversity. These findings highlight historical biogeography of *I. alpestris* in Slovenia and underscore the need higher-resolution genomic analyses to refine our understanding of past and present population dynamics.

ECOLOGY

Hydric traits across spatial and temporal gradients: Changes in water loss across seasons and the fog moisture gradient in the Namib day gecko, *Rhoptropus afer*

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Abstract

We studied seasonal and spatial differences in Evaporative Water Loss (EWL) in a Namib Day Gecko (*Rhoptropus afer*) over a 10-month period. Terrestrial reptiles face the challenge of maintaining a stable hydric state in the variable environments they inhabit. When water availability changes temporally (across seasons), seasonal physiological plasticity (acclimatisation) can facilitate homeostasis in changing environments. This has been widely studied for thermobiology and metabolism. However, EWL of reptiles in response to changing water availability is still poorly studied. Even in extremely arid environments, such as hyperarid deserts, seasonal fluctuations in humidity (and precipitation) affect hydric homeostasis and exert physiological pressure on the organisms living there. *Rhoptropus afer* is a 3.5 g diurnal gecko with very small home ranges on bare granite rocks. We measured EWL over a period of nine months of increasing aridity, followed by a rain event and the month after the rain. EWL was significantly higher in the driest month of the season (March), immediately before the rain event, and decreased in April, after the rain. It is possible that the seasonal changes in EWL represent an alternative physiological adaptation that precludes the benefits of a fixed relationship between EWL and aridity. We also studied three populations across a spatial moisture gradient during the dry season (January and February). Sites differed in relative humidity and the number of fog days per year (*R. afer* is known to lick dew to obtain water). EWL differed between the site

with the middle number of fog days and the other two sites, suggesting adaptation to local moisture conditions, but may also be related to changing food availability as the site is surrounded by perennial, green vegetation. These results suggest that the plasticity of EWL is an adaptive response to immediate seasonal or local conditions, which could be further explored with common garden experiments.

Symposium

Ecology and evolution of amphibian larvae

Organisers: Pedro Henrique dos Santos Dias & Paul Lukas



ECOLOGY

Performance and behavioural responses of *Bufo spinosus* to temperature-diet interactions

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Abstract

Ectotherms are vulnerable to warming, as their metabolic rates scale strongly with temperature, influencing their physiology and behaviour. Dictating the energetic and nutritional requirements of ectotherms, temperature may interact with diet, affecting performance, possibly resulting in dietary shifts to increase energetic efficiency or to maximize specific traits. We investigated the combined effects of temperature and diet on the performance and dietary choices of tadpoles of the Spiny toad (*Bufo spinosus* Daudin, 1803). We reared tadpoles at 12°C, 16°C and 20°C and fed them with

animal, plant, or mixed diets (both). We compared responses in the life-history traits (survival, growth rate, larval period, body mass, length and condition), and used stable isotope analysis to quantify the diet assimilation of tadpoles on the mixed diet. Temperature impacted all traits, which were maximized in different regions of the nutritional state-space composing the multidimensional fitness landscape. These trait-specific effects were stronger effects on larval period and growth rate. Warming accelerated growth rate and development, reducing body mass and condition, while cold prolonged the larval period allowing for greater weight and condition, but not length. Toadlets from the animal diet had higher weight and condition at 12°C than those on the plant diet, but at 20°C they performed equally in both traits. Tadpoles allowed to feed selectively coped efficiently with temperature effects on growth rate, as well as on body mass and length, outperforming tadpoles fed on the single diets. This reveals adaptive feeding choices, as confirmed by the changes in diet assimilation, which tended towards greater herbivory with increasing temperature. A better understanding of the general trends in temperature-diet interactions in ectotherms will be critical to develop realistic predictions of the ecological impacts of climate change on species and ecosystems.

CONSERVATION

Promising Beginnings, Hopeful Returns: Experiences with Population Reinforcement for *Triturus carnifex* in Jovsi (Slovenia) through Supportive Breeding within the LIFE AMPHICON Project

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Abstract

The *Triturus carnifex* population in Jovsi (Natura 2000 Dobrava–Jovsi) is small, isolated, and therefore highly endangered. Its decline has been driven by habitat loss due to changes in water regime, agricultural intensification or land abandonment leading to overgrowth. Historically, regular flooding in the area maintained high water levels and suitable breeding conditions. In recent years, worsened by climate changes, water bodies have dried up before completion of metamorphosis, increasing the risk of local extinction. To counter this, the LIFE AMPHICON project introduced supportive breeding to significantly improve egg and larval survival and maximize metamorphosis success, providing an immediate boost to the population and an effective response to species decline. From 2022 to 2024, 427 juveniles were successfully reared from 1113 eggs and released to Jovsi, with an overall success rate of 38%. Mortality occurred mostly at the egg stage, presumably due to a chromosome No.1 syndrome, while larval survival in the breeding station was nearly 100%. Daily inspections provided valuable data on growth and development. Shortly after metamorphosis, we photographed the ventral patterns of individuals for later identification and observed notable but rarely documented changes in patterning over 2–4 weeks. While supportive breeding provides essential support to endangered populations, the long-term survival of newts depends on comprehensive habitat restoration and ongoing management. The aim is to build a stable, self-sustaining population supported by both suitable water and terrestrial habitats. Therefore, we excavated 10 ponds and a management plan is being implemented to restore the surrounding land into extensive wet meadows. After promising beginnings with high breeding success and fast natural colonization of water-retaining ponds, the recapture of reared individual confirms successful hibernation and marks a hopeful return of the conservation effort.

EVOLUTION

Something old, something new, something borrowed – the evolution of the anuran larval oral apparatus**Pedro Henrique dos Santos Dias**

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One of the major goals of evolutionary biology is to explain how novel traits arise. Evolutionary novelties are the engine of adaptive radiation, allowing organisms to expand into new adaptive zones and to colonize new niches. The central role of novelties in the evolutionary and diversification processes can be observed in the tree of life, where major cladogenetic events are associated with the acquisition of novel character states. Although we can list many examples of astonishing evolutionary novelties, our knowledge regarding the ability of organisms to innovate and the processes involved is particularly limited. The anuran larval oral apparatus is a case of such a poorly understood novelty. The oral apparatus, also known as the oral disc, is a transient feature composed of soft and keratinized structures. It allowed frogs to explore all freshwater environments and to occupy different niches, exploring a variety of trophic resources. Detailed, comparative anatomy and development, associated with gene expression patterns revealed an intriguing evolutionary history, in which a novel sensory system and the co-option of ancient regulatory networks explain the rise of this unique novelty.

CONSERVATION

The microbiome as a link between diet quality, health, and escape behavior in amphibian larvae under heat stress

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Abstract

What animals eat is fundamental to their growth and development, and one of the main factors modulating their microbiomes. Microbiomes adjust to environmental conditions and influence host physiology and behavior. Thus, they can improve the host's ability to deal with environmental stressors, such as those resulting from climate change, as increased temperatures and altered food quality. We conducted a multifactorial experiment to test whether three diets with increasing amounts of protein, fat, and components of animal origin (designated low-, intermediate- and high-quality, respectively), two rearing temperatures (18 C or 24.5 C), and exposure or not to a heat wave (28 C for 48h) shaped the gut bacterial community of tadpoles (*Rana temporaria*). We then examined how the treatments, associated shifts in bacterial communities, and predicted metabolic pathways were related to host nutrient assimilation (isotopic signatures), health (body condition and developmental rate), and escape behavior. Tadpoles maintained their body condition and developed faster at 24.5 C and with increasing diet quality (reduced herbivory) at this temperature. However, high-quality food may not be available ad libitum in the wild, making an herbivorous diet the best available option for ectotherms shown to prefer herbivorous diets under increased temperatures. The intermediate-quality diet resulted in tadpole decreased ability to react to an aversive stimulus at 24.5 C, but not in the group exposed to the heat wave. The heat wave may have triggered an increase in *Klebsiella* abundance, with concomitant increase in the myo-inositol degradation pathway, which plays a role in controlling cell membrane fluidity and signaling and may increase attention levels. Similar outcomes in host performance under most experimental conditions highlight the existence of plasticity of the bacterial community and

alternative enterotypes with functional redundancy that are compatible with host health.

EVOLUTION

Main environmental drivers of tadpole shape and color variation

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Abstract

Amphibians have evolved the greatest diversity of reproductive modes across tetrapods. Within this diversity, a large fraction of anuran species maintains complex life cycles including a larval stage. In accordance with the diversity of microhabitats that anuran larvae have specialised in, we observe remarkable variation in tadpole size, shape and pigmentation across species. Inevitably, a lot of this phenotypic variation is the joint result of genomic variation and environmental induction. To understand to what extent such phenotypic variation in anuran larvae may be originated through developmental responses to environmental factors, we have experimentally exposed larvae of Western spadefoot toads (*Pelobates cultripes*) to factors affecting their growth, development and pigmentation: risk of predation, risk of pond drying, and varying background colour. Phenotypic responses to these factors individually resulted in altered size, body shape, pigmentation and time to metamorphosis, reproducing in a single experiment the range of phenotypic variation observed throughout the entire distribution range of the species. Moreover, when these factors were crossed, we observed interactive and at times conflicting phenotypic responses of the larvae, suggesting complex regulation of these developmental responses resulting in a fine-tuned adjustment to the local conditions experienced.

EVOLUTION

Embryonic development of South American frogs: New insights into the evolution of the hyloids *Neoaustrarana* and *Telmatobiidae*

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Abstract

Hyloidea comprises the majority of frog species worldwide. It has been suggested that its early evolution took place in southern South America, followed by a radiation into the continent's tropical regions. *Neoaustrarana* is the sister group to all hyloids except *Rhinodermatidae*, while *Telmatobiidae* is the sister taxon to all remaining hyloids, the *Cornucopirana* clade. In this study, we present new developmental data for *Neoaustrarana* and *Telmatobiidae*, integrating clutch structure, morphological descriptions, hatching behavior, and developmental series within a phylogenetic framework. Hyloidea exhibits a wide variety of reproductive modes, including the rare case of exoviviparity in *Rhinodermatidae*. Within *Neoaustrarana*, eight different clutch types are present, but ancestral state reconstruction recovers an aquatic clutch and exotrophic development. In *Rhinodermatidae* and in most species of *Neoaustrarana*, clutches lack a common jelly matrix, and although optimization is ambiguous, it is

tempting to suggest that clutches embedded in a collective jelly matrix may represent a derived condition for Hyloidea. Optimization also reveals that late hatching is a synapomorphy of Rhinodermatidae and of non-aquatic breeders within Neoaustarrana. These species, regardless of whether they have exo- or endotrophic development, share an early onset of hindlimb development. Moreover, most embryos with late hatching lack adhesive glands and have more than 50% of yolk proportion. Variation in hatching moment is also widespread in Neoaustarrana and represents the ancestral condition of Hyloidea, with a shift in the derived clade Cornucopirana. Owing to the key phylogenetic position of Neoaustarrana and the limited availability of developmental series in both collections and literature, this study offers essential insights into the evolutionary history of Hyloidea.

OTHER

Exposure of different stages of tadpoles to light pollution

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Abstract

Amphibians are the most threatened vertebrate group and are susceptible to numerous environmental changes. One of them is a disturbance in normal circadian rhythm due to the exposure to artificial light at night (ALAN). We investigated the effects of two different intensities (low and high) of two different types of LED lights (warm and cold) on the development of green frogs (*Pelophylax kl. esculentus*) compared to the control group. We compared the effects of light at different developmental stages. In the first round of experiments, tadpoles were at GS 20 at the beginning of the experiment, and in the second, at GS 25. Control group was exposed to artificial daylight and night cycle, while experimental groups were exposed to artificial daylight, and different types and intensities of ALAN. After the experiment, energy budget (protein and lipid concentration) and biomarkers activities (AChE, GST, CAT) were investigated. Our results show that tadpoles that were at higher developmental stages were more susceptible to the effects of ALAN. The most pronounced impact was observed under higher intensities of cold LED light, while negative impact was recorded in some of other treatments as well; i.e. all treatments had significantly lower activities of catalase. Energy budget showed increased protein levels in treatments with higher ALAN intensities. These data provide an insight into physiological consequences that could arise from exposure to ALAN and more attention should be given to light pollution in urban areas where amphibians are present.

EVOLUTION

Species-Specific Chemical Communication in Tadpoles: Ecological Contexts and Alarm Cue Composition

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Abstract

Chemical communication is widespread among animals but remains underexplored across a wide range of anuran taxa and ecological contexts. To address these gaps, we expanded research on chemical communication in predator–prey interactions using bioassays of four anuran species with divergent life histories, with a particular focus on poison dart frogs (Dendrobatidae): *Ranitomeya sirensis*, *Epipedobates anthonyi*, *Bufo bufo*, and *Rana temporaria*. We also sought to characterise the physicochemical properties of alarm cues. Tadpole bioassays revealed species-specific responses: *R. sirensis* responded only to conspecific alarm cues, while *R. temporaria* reacted to both con- and heterospecific cues. In contrast, *E. anthonyi* and *B. bufo* showed weak or no responses. These patterns likely reflect ecological traits such as parental care, defensive strategies, and social structure. Physicochemical analyses indicated that alarm cues likely consist of small, stable, non-proteinaceous compounds. An amino acid elicited antipredator responses in *R. temporaria* but not in *B. bufo*, supporting the hypothesis that these substances may function as ecologically relevant cues (passive metabolic by-products) rather than evolved signals. Variation in behavioural responses based on stimulus preparation methods further highlights the need for standardised protocols. Overall, the results suggest that chemical communication in frogs is more diverse and context-dependent than previously assumed, with implications for sensory ecology. This research advances understanding by addressing gaps in taxonomic coverage, ecological function, and methodological consistency.

EVOLUTION

No bones about it: A comparative dive into tadpole cartilage development

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Abstract

The cartilaginous skull (chondrocranium) of larval anurans exhibits remarkable developmental and morphological diversity shaped by phylogenetic history and ecological specialization. Comparative studies across species such as *Xenopus laevis*, *Bufo bufo*, *Bombina orientalis*, and *Discoglossus scovazzi* reveal a largely conserved sequence of cranial cartilage formation, while taxa like *Hymenochirus boettgeri* and *Lepidobatrachus laevis* demonstrate pronounced deviations linked to feeding strategies and heterochrony. Arboreal species such as *Smilisca phaeota* and *Staurois parvus* show further modifications according to the life strategy. By analyzing the timing and order of chondrification events—particularly in elements like Meckel's cartilage, the ceratohyal, and palatoquadrate—this research highlights both conserved modules and lineage-specific innovations. These patterns offer insight into how developmental plasticity contributes to anuran cranial evolution.

ECOLOGY

The effects of biofilm-colonised microplastics on the growth and developmental rates of amphibians

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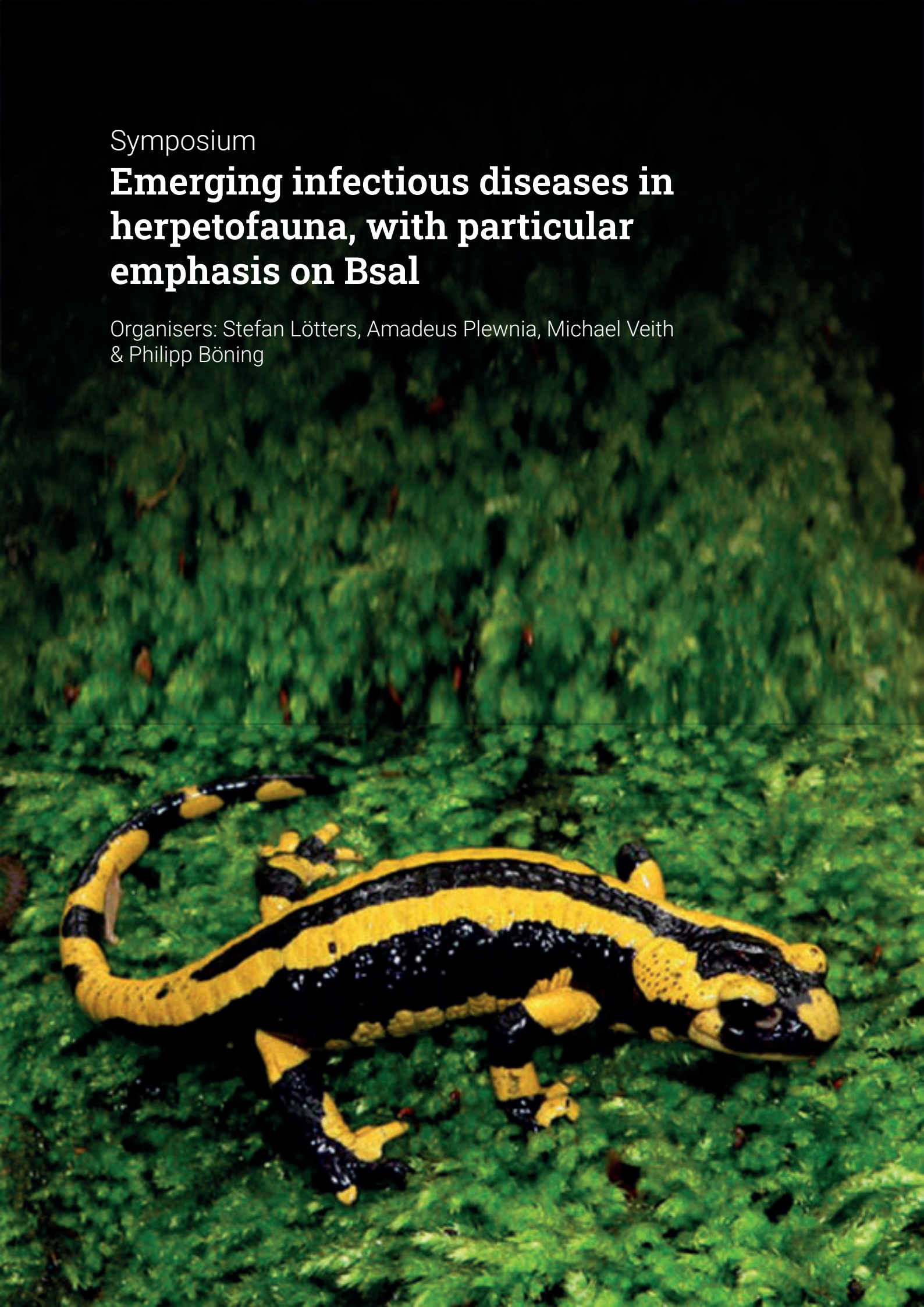
Abstract

Microplastics (MPs) are widespread in aquatic environments, and biofilm formation on their surfaces can alter their physicochemical properties and biological effects. Amphibians are potentially susceptible to ingesting biofilm-covered MPs due to their complex life cycles and non-specific feeding modes. Yet, whether such exposure includes physiological consequences remains poorly understood. In this study, we exposed larvae of the Western spadefoot (*Pelobates cultripes*) to pristine and biofilm-covered polyethylene MPs –with ground mussel shell as a natural particle control– to assess effects on growth and developmental rates during larval and juvenile (i.e., post-metamorphosis) stages at two temperatures (23°C and 26°C). Our findings show that particle type had no significant effect on either developmental or growth rates. Temperature significantly influenced both traits, with larvae and juveniles developing slower at 23°C than at 26°C. The results provide evidence that the ingestion of pristine and biofilm covered MPs does not directly alter amphibian growth or development. Given global amphibian declines and the continuous entry of MPs into the environment, understanding both direct and indirect MP effects is essential for assessing the risks they pose to amphibian health. Ongoing analyses will assess the multi-level effects on gut health and the accumulation of MPs in different organs.

Symposium

Emerging infectious diseases in herpetofauna, with particular emphasis on Bsal

Organisers: Stefan Lötters, Amadeus Plewnia, Michael Veith
& Philipp Böning



DISEASES

Finding the best way to detect and confirm amphibian pathogen

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Abstract

Over two decades of intense research in the field of amphibian emerging infectious diseases has led to identification of several globally important pathogens and has generated vast array of diagnostic methods. Some of the procedures gained universal popularity and renown, while many were not widely recognised, partially due to general focus on few selected pathogens and possibly also by lack of comprehensive summary of possible available diagnostic approaches. We summarized available assays used in molecular detection and typing of amphibian pathogens based on DNA amplification including conventional PCR, nested PCR, qPCR and LAMP. The list contains 60 different sets aimed at multiple pathogen taxa with various level of analytic specificity. The presented technical details for detection of pathogen species/lineage include target gene, product length, primer and probe sequences, concentrations, thermocycling conditions and the original source with notes on its recognition. We discuss inconsistency in methodology reporting, with not all articles providing all necessary assay details and cases when focus on DNA diagnostic proved misleading. We point out the most common issues when adopting a new method and sum up suggestions on how to deal with them. We hope our effort will help to expand the scope of amphibian disease research beyond the infamous chytridiomycosis and assist new researchers in diversification of diagnostic approaches even with basic laboratory equipment. Keywords: Amphibian disease; Amphibian Herpesvirus; Batrachochytrium dendrobatidis; Batrachochytrium salamandrivorans; Chytridiomycosis; Chlamydia, Diagnostic assays; Emerging infectious diseases; LAMP; pathogen pollution; PCR; Perkinsea; qPCR; Ranavirus; Real-time PCR

DISEASES

Omnipresent herpesvirus infections in common toads (*Bufo bufo*) do not correlate with population declines

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Abstract

Emerging infectious diseases are one of the major drivers of the current worldwide amphibian decline. Bufonid herpesvirus 1 (BfHV1) was recently described in 2018 in Switzerland following a mass-mortality episode in one common toad (*Bufo bufo*) population. Since then, the disease has been reported in the UK and Germany. We studied the potential role of the emergence of BfHV1 in widespread, enigmatic toad declines in Belgium. During the spring migration of 2024 we sampled 30 common toads from 10 declining and 30 from 10 non-declining populations, comparing body condition and herpesvirus infection prevalence. BfHV1 compatible lesions in live and road-killed toads and PCR analysis of livers of road-killed toads were used to estimate prevalence. BfHV1 occurred in all study populations (average 30.8% infected animals per location) and we did not find any correlation between the presence of the virus, the toad's body condition, herpesvirus morbidity and population status. The lack of obvious effects of an omnipresent, largely subclinical infection, however, does not exclude more subtle negative, effects that may warrant further research.

DISEASES

From outbreak to action: multidisciplinary research on batrachochytrid threats to amphibians in the Netherlands

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Abstract

The discovery of *Batrachochytrium salamandrivorans* (Bsal) took place roughly fifteen years ago in the Netherlands, where this chytrid fungus decimated local fire salamander (*Salamandra salamandra*) populations. In recent years, Bsal has been detected at several other sites across the country where it affects crested newts (*Triturus cristatus*). The threat that Bsal and its congener *Batrachochytrium dendrobatidis* (Bd) pose to national and European protected amphibian species, and biodiversity as a whole, was recognized by the Dutch government. As a result, a multi-year research project was initiated in which focus lies on i) quantification of Bsal impact on newt populations; ii) exploring how amphibian assemblage composition may influence pathogen presence and prevalence ; iii) disentangling relative influence of environmental factors on local Bsal disease dynamics; and iv) assessing Bsal-Bd coinfection risk. These research activities are being performed as a collaborative effort between the Dutch NGO RAVON and Belgian partner Wildlife Health Ghent of Ghent University. The ultimate goal of the project is to develop concrete management and conservation strategies that facilitate amphibians in gaining the upper hand over continually present chytrid fungi.

DISEASES

A decade of the salamander plague in Germany

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Abstract

The ongoing amphibian crisis is the result of a multitude of factors, with emerging infectious wildlife diseases playing a key role. One of these is the salamander plague, caused by the chytrid fungus *Batrachochytrium salamandrivorans* (Bsal) affecting primarily salamanders and newts. Bsal is native to Asia and invasive in Europe. Here, Bsal outbreaks are known in the wild from > 120 sites in Belgium, Germany, Spain, and the Netherlands. First discovered in 2010, the salamander plague mainly affects the European fire salamander, in which it causes dramatic population declines with mass mortalities. Despite its virulence, it remains unsolved why fire salamanders still exist at outbreak sites and in some cases reproduce. Explanations range from previously uninfected animals to possible resistance. For other caudates, even less is known about the impact on all ecological scales. While the decline of the northern crested newt may be related to Bsal, the other newts are considered tolerant and thus may act as Bsal reservoirs and vectors. The Alpine salamander so far seems unaffected by Bsal spread. Germany is still a Bsal 'hotspot' with 90% of all sites, located in four federal states. Bsal regions include the northern and southern Eifel (detected in 2015 and 2017 respectively); the Ruhr area (detected in 2017) with the adjacent regions; the Steigerwald (detected in 2020); Swabia (detected in 2020) and more recently, Hesse (detected in 2024). It is undisputed that Bsal is spreading further, but dispersal speed and mechanism remain little understood. Besides amphibians, other wildlife and humans could play a role as vectors. However, the current occurrence of the salamander plague could also be due to independent introductions. Given the many uncertainties and open questions we face after a decade of the Bsal invasion in Europe, it is evident to invest more resources into science and surveillance to avert further biodiversity loss driven by Bsal.

DISEASES

Ecological drivers and behavioural traits of ophidiomycosis in dice snakes (*Natrix tessellata*) from Northern Italy

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Abstract

Ophidiomycosis, caused by the fungus *Ophidiomyces ophidiicola* (Oo), is an emerging infectious disease threatening wild snake populations globally, but its ecology and impacts in Europe remain poorly understood. We investigated a dice snake (*Natrix tessellata*) population from the northern shore of Lake Como, Italy, through standardised surveys conducted across an entire active season, aiming to assess the prevalence of Oo, characterise the circulating strain, and identify key ecological and behavioural correlates of Oo presence and ophidiomycosis.

Snakes were screened for Oo using qPCR and histopathological analyses, coupled with environmental, morphological, and behavioural assessments. Molecular

diagnostics identified a single Oo strain (i.e., Clade II). Oo prevalence was remarkably high, and the occurrence of clinical signs was strongly associated with positive diagnostic results. Juveniles and smaller snakes exhibited increased susceptibility compared to larger individuals, suggesting potential age-related or immune-driven vulnerability.

Infected snakes displayed significantly lower body temperatures than uninfected ones, indicating a possible physiological impairment or reduced thermoregulatory efficiency. Interestingly, despite reduced body temperatures, Oo-positive snakes did not preferentially select warmer substrates, suggesting limited or absent behavioural fever as a defensive mechanism. Moreover, although body condition was not significantly affected by Oo presence or ophidiomycosis, positive snakes showed altered defensive behaviours, notably a significant reduction in musking responses, potentially compromising their ability to deter predators.

Our results suggest complex ecological interactions between Oo and host behaviour in dice snakes, highlighting the necessity for multi-year capture–recapture studies to fully understand disease dynamics, long-term impacts, and possible host adaptation to ophidiomycosis in Europe.

DISEASES

Urodeles under threat: on the impact of the chytrid fungus Bsal on northern crested newt (*Triturus cristatus*) populations

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Abstract

The emerging pathogen *Batrachochytrium salamandrivorans* (Bsal) has been present in Europe for at least two decades where it is widely documented to cause mass mortality and population declines, but not extirpation, in fire salamanders (*Salamandra salamandra*). Population impacts of Bsal on other European urodeles however remain poorly investigated and, without intensive monitoring, large-scale population declines may go unnoticed. Here, we present initial results on population responses in the northern crested newt (*Triturus cristatus*), a highly susceptible Bsal host in ex situ infection experiments, through infection histories in newts marked over the last five years at a Bsal outbreak site in Gorssel, the Netherlands, and a disease-structured multistate mark-recapture model. Bsal is a seasonal, enzootic pathogen in *T. cristatus* in Gorssel, with a high springtime prevalence and high pathogen loads. Fluctuating thermal conditions during the aquatic phase, combined with long infection timespans,

are likely decisive factors in determining population responses to Bsal in this species. These results encourage further research on the impact of environmental factors on Bsal-host dynamics.

DISEASES

Ten years of blood parasitism data for six lizard species from Iberia, the Canary Islands, and Morocco

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Abstract

Parasites can affect major physiological host traits, with potential impact on their populations. Yet, how parasite infections change across time and space is poorly understood, especially in reptile hosts. Combining microscopy and molecular data, we assessed blood parasites in three biogeographic contexts – an Atlantic climate in Northern Portugal, a Mediterranean in the Atlas Mountains of Morocco, and a subtropical in the Canary Islands – for 6 lizard species (5 lacertids and 1 gecko) over an interval of approximately 10 years, with extensive almost biennial data for *Gallotia galloti* from Tenerife. We assessed how blood parasites are related to lizard morphological traits, microclimate, and ectoparasite data (when available). Our results show body size (snout-vent length) and condition are often positively significant for parasite infection, although which of these traits is significant is species specific, suggesting specific host-parasite idiosyncrasy. Males are often expected to be more parasitized, but this was only observed in 1 species, suggesting sex-related exposure to parasites vary depending on ecological context. We detected annual fluctuations in blood parasite infection (climatic or host demographic-related), but often no seasonal effects. Increasing ambient temperature is positively associated with blood parasite prevalence in Portugal and Tenerife, but negatively in the Atlas Mountains, suggesting altitudinal adaptations by parasites or vectors might be modulating these dynamics. The pattern of change and factors that influence blood parasite prevalence and intensity also differ across host-parasite systems, suggesting different factors promote infection contraction and growth. Hematophagous mites are known vectors for certain blood parasites, but we found no association with blood parasite infection. Local conditions and co-adaptive histories of each system are important to understand host-parasite dynamics and need to be researched separately.

DISEASES

Co-Exposure with the Herbicide 2,4-D Does Not Exacerbate *Batrachochytrium salamandrivorans* Infection in the Italian Crested Newt (*Triturus carnifex*)

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Abstract

Amphibians face a multitude of threats and therefore make a prime example of the current biodiversity crisis. Multiple amphibian stressors in anthropogenic landscapes include infectious diseases and agrochemicals. Synergic effects between these stressors may increase the negative impact of infections on amphibian health. In a 56-day trial, we assessed the impact of co-exposure to the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and the pathogenic fungus *Batrachochytrium salamandrivorans* (Bsal) on infection parameters (infection intensity and disease severity) and health (body condition and telomere length) in Italian crested newts (*Triturus carnifex*). Twenty days post-inoculation with Bsal, newts were either exposed to 2,4-D for 12 days or not exposed (control). Most newts developed high infection loads that steadily increased towards the end of the trial. While body condition was negatively correlated with pathogen burden, only one out of 23 animals died. Telomere length remained unaffected by the pesticide and the pathogen. The 2,4-D treatment

did not exacerbate Bsal infection. Most newts survived almost two months with significant pathogen loads; thus, even in a pesticide-infested environment, *T. carnifex* could be an important long-term Bsal reservoir for co-occurring species on the Italian peninsula, a urodele diversity hotspot.

DISEASES

Multi-omics approaches to investigate host response to Bsal exposure and determinants of chytridiomycosis severity in *Triturus carnifex*

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Abstract

In the midst of the current biodiversity crisis, amphibians are severely threatened by emerging diseases such as the deadly chytridiomycosis. Characterizing the mechanisms that underly susceptibility to this disease is therefore fundamental to improve amphibian conservation. Using a comprehensive multi-omics approach, we investigated the impact of an exposure to the deadly chytrid fungus *Batrachochytrium salamandrivorans* (Bsal) on the gene expression of Italian crested newts (*Triturus carnifex*) and on the bacterial symbionts that constitute their skin microbiota. Exposure to Bsal affected multiple components of the newts' immunity, from structural changes in their microbiota, to the activation of the inflammasome and development of adaptive immunity at the site of infection (skin) and in their primary lymphoid organ (spleen). Bsal exposure was also characterized by a suppression of translation processes and of skin keratinization. Within Bsal-exposed newts, chytridiomycosis severity was positively correlated with the epidermal and splenic enhancement of stress responses, reduction of basal metabolism and dysregulation of tissue integrity. Together, our results suggest that in *T. carnifex*, Bsal susceptibility may be linked to the reprogramming of energy resources from the maintenance of basal metabolism and tissue integrity, towards elevated immunity and tissue restructuring. This study sets ground for future research on the determinants of variation in susceptibility to the deadly chytridiomycosis, and opens new perspectives for amphibian conservation.

CONSERVATION

Development of the “Fire Salamander Ark Project” in Wuppertal - an interdisciplinary in situ and ex situ species conservation program to save the local fire salamander population

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Abstract

The fire salamander (*Salamandra salamandra*) is threatened by *Batrachochytrium salamandrivorans* (Bsal), which spread to Wuppertal, Remscheid, and Solingen in 2020, resulting in mass mortality events. In response, the “Fire Salamander Ark Project” was launched in March 2021 by Zoo Wuppertal and the University of Wuppertal, together with numerous cooperating partners. The project combines in situ and ex situ measures, including larval monitoring, habitat restoration, and environmental DNA testing. For the latter, standardized protocols are being developed as part of a doctoral thesis. Wild salamanders are tested for Bsal and, if infected, treated using thermal therapy (25°C for 14 days) in specific incubators. Post-treatment observation for six weeks and repeated testing are conducted before animals enter an ex situ breeding program, which aims to preserve genetic diversity and enable the future release of offspring into safe habitats. The project also promotes public education through lectures, print and online publications, a virtual learning platform and 3D-printed, hand-colored salamander models, based on specimens from the

Zoological Research Museum Alexander Koenig. We thank Benny Trapp (NABU Wuppertal), Till H. Iseke (Kalkwerke H. Oetelshofen), and all other project partners for their contribution and the Friends of Zoo Wuppertal Association, Ingo and Waltraud Pauler Fund of DGHT, and Stiftung Artenschutz for their financial support. Overall, the Fire Salamander Ark Project is a strong and successful example of how zoos, universities, and multiple stakeholders can work together across disciplines to protect an endangered species and its habitat both in situ and ex situ.

DISEASES

The Kruppwald fire salamanders - A spark of hope?

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Abstract

Since its description in 2013, Germany has become the centre of distribution for the chytrid fungus *Batrachochytrium salamandrivorans* (Bsal). Here it has been detected in four federal states, with hotspots in North Rhine-Westphalia.

The introduction of Bsal into fire salamander (*Salamandra atra*) populations typically results in mass mortality and subsequent strong declines. However, some populations persist over longer periods. In the Kruppwald (Essen, North Rhine-Westphalia, Germany), the first infections and Bsal-related mortalities in fire salamanders were reported in 2019. Yet, while two neighbouring populations were largely extirpated by Bsal-related mass mortalities, the Kruppwald population persists in notably higher population numbers to the present day.

In our study, we analysed long-term Bsal prevalence and capture-recapture data of the Kruppwald fire salamanders over almost eight years. Through our capture-recapture studies, we confirmed six instances of individuals that survived without infection over

three or more years. Two individuals that were tested positive for Bsal were later tested negative. We further found significant differences in larval densities between the Kruppwald and the two adjacent populations, with the Kruppwald showing a 44 times higher larval density in comparison. Our findings raise several further questions about whether the observed persistence of the Kruppwald population might be due to some kind of resistance against Bsal, or instead to specific habitat or metapopulation characteristics.

A central question remains whether the recaptured Bsal-positive individuals are the result of potential increased resistance or rather a result of chance effects, since no clinical signs were observed on these animals' skin.

Further detailed investigations in the Kruppwald are required to determine how resilient wild *S. Salamandra* populations are against Bsal outbreaks.

DISEASES

Pathogen detection in larval and post-metamorphic amphibians in northern Germany

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Abstract

Emerging infectious agents – including the well-described *Batrachochytridium dendrobatidis* (Bd) and *B. salamandrivorans* (Bsal), ranaviruses, and the recently documented bufonid herpesvirus (BfHV1) and ranid herpesvirus (RaHV3) – are associated with disease outbreaks in amphibian populations worldwide.

In Schleswig Holstein, BfHV-1 and RaHV-3 have been recently detected in native amphibian populations. However, infectious disease studies in the area remain scarce. To monitor the occurrence of multiple infectious agents, skin swabs from adult and subadult individuals including 150 water frogs (*Pelophylax* spp.), 48 moor frogs (*Rana arvalis*), 40 common toads (*Bufo bufo*), and 32 common frogs (*Rana temporaria*) were collected in four locations in Schleswig Holstein in 2025. Additional swabs from 36 moor frogs and 35 common frog tadpoles were collected at two locations, targeting keratinized body parts according to Gosner developmental stage (Gosner 1960). All swabs were analyzed via PCR for Bd, ranaviruses, BfHV-1, and RaHV-3.

Prevalences varied significantly between species (p -value < 0.05). Bd was detected in 14.7% of water frogs and 2.5% of common toads. 75% of common toads and 9.4% of common frogs tested positive for BfHV-1, while RaHV-3 was found in 40.6% common frogs. Interestingly, 11.4% of common frog tadpoles tested positive for RaHV-3. No ranaviruses were detected in any of the samples. Analyses showed significant spatial

variability in pathogen detection across sampling sites, likely influenced by local environmental conditions and host community variations.

This study confirms the presence of emerging infectious agents across different native amphibian species and life stages in northern Germany. However, their impact in amphibian populations, and the specific role of the different host species in pathogen transmission and persistence requires further research.

DISEASES

New insight into the effects of the *Strigea robusta* parasite on abnormalities in amphibians

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Abstract

Various developmental abnormalities are common among amphibian populations. Some of them manifest themselves in the form of morphological deformations incompatible with life. One of the longest studied cases of amphibian polymorphic syndrome in Europe is anomaly P, caused by the trematode *Strigea robusta*. The anomaly P represents a unique instance of host-parasite interaction since the parasite's influence alters the strictly regulated complex morphogenesis of vertebrates. The effects of this parasite include symptoms such as polydactyly, brachymelia, and additional limbs. Such deformities often reduce the locomotor activity of amphibians in order to complete the life cycle of the parasite. Moreover, the *S. robusta* hyperinvasion can lead to mass mortality in amphibian populations.

In this paper, we present the results of our recent research devoted to this anomaly. Not all amphibian species exhibit deformations when exposed to trematodes. Experimental studies we conducted have shown that *S. robusta* does not induce abnormalities in *Dyscophus antongilii*, *Xenopus laevis*, *Rana temporaria*, and *R. arvalis* even at the highest doses of cercariae (48), whereas alterations in *Pelophylax* were noted at doses of 2-4 cercariae. We also recorded a slight divergence among the trematodes parasitizing different host mollusks (*Planorbis* and *Planorbarius* lineages). The different genetic lineages of the parasite had no differences in the effect on the deformations induction in amphibians. We discovered that it is a stage-dependent process; after specific limb formation stages, no abnormalities are observed among tadpoles exposed to trematodes. The range of this parasite and, as a result, the place of appearance of the anomaly is much wider than it was previously known: in recent studies, they were found in Europe and Penza and Middle Volga regions of Russia. Lately we have identified *S. robusta* in the Russian Far East and West Siberia, which makes the anomaly's area transcontinental.

DISEASES

Parasite Island: Drivers of Ectoparasite Levels in Insular Lizard Populations

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Abstract

Parasites are key drivers of ecological and evolutionary processes, yet the factors shaping ectoparasite levels in insular populations remain poorly understood. We studied ectoparasite (mite and tick) loads in three lizard species — *Mediodactylus kotschyi*, *Hemidactylus turcicus*, and *Podarcis erhardii* — across 83 island populations in the Aegean Sea, Greece. We captured animals in the field, measured their morphology and physiological condition, as well as quantified their ectoparasites. We tested hypotheses relating parasite burdens to environmental variables (grazing intensity, resource availability, marine subsidies), host traits (sex, size, pregnancy, tail condition), and physiological responses (body condition, body temperature). Our results show that parasite loads vary substantially between species: *M. kotschyi* carried the highest mite loads, *P. erhardii* the highest tick loads, while *H. turcicus* had the lowest levels overall. Larger individuals tended to host more mites, though tick loads were size-dependent only in *M. kotschyi*. Surprisingly, parasite loads showed few consistent links to sex or pregnancy. Grazing intensity was associated with increased tick loads in *M. kotschyi*, but marine subsidies and arthropod biomass had limited effects. Individual-level analyses revealed that mite load correlated with body condition and tail loss in *M. kotschyi*, but parasite loads did not consistently reduce body condition across species. Our findings suggest that host-parasite interactions on islands are shaped by a complex interplay of species-specific traits, individual health, and environmental context, emphasizing the need to integrate ecological, physiological, and biogeographical perspectives in island parasitology.

CONSERVATION

To trap or not to trap: effectiveness of different heated refuges against chytridiomycosis in tadpoles

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Abstract

Chytridiomycosis, an amphibian disease caused by the fungus *Batrachochytrium dendrobatidis* (Bd), has contributed to global amphibian declines and extinctions in the last five decades. The pathogen's thermal maximum is below that of most host species, making thermal treatment a potential mitigation strategy. In this study, we investigated whether providing heated refuges could reduce Bd prevalence and infection intensity in common toad (*Bufo bufo*) tadpoles kept under semi-natural conditions. We exposed tadpoles to Bd and housed them in 100-L mesocosms (10 tadpoles per unit) with no refuge (control) or one of eight refuge types, either heated (30 °C) or unheated over an eight-day period. Refuges varied in design: some had bottom openings, lateral openings, or both, and included both freely accessible refuges and those that entrapped tadpoles. After treatment, tadpoles were weighed, euthanized, and Bd infection intensity was assessed by qPCR. Mortality remained low throughout the experiment. The presence of heated refuges did not reduce Bd prevalence in any treatment group. Tadpoles in mesocosms with trap-like refuges had slightly lower infection intensities than controls or those in more open refuges, but this effect was not statistically significant. Water temperatures were elevated in mesocosms with lateral-opening refuges, which correlated with increased tadpole

body mass. Trap-like refuges did not lower the body mass of individuals. These findings suggest that the tested refuge designs are insufficient to effectively mitigate Bd infection in common toad tadpoles and may incidentally alter the thermal environment of aquatic habitats. Further research is necessary to refine this approach for efficacy and environmental compatibility.

DISEASES

Elucidating the enigmatic absence of Bd in the Colombian Sierra Nevada de Santa Marta mountains – a serious conservation concern

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Abstract

Amphibians are among the most threatened vertebrate groups, with the chytrid fungus *Batrachochytrium dendrobatidis* (Bd) being a major driver of declines. On a global scale, Neotropical highlands are among the regions most heavily affected by the pathogen due to optimal conditions for Bd growth. Here, declines have been documented since the 1970s, with virtually no montane region thought to be spared from Bd invasion. The Sierra Nevada de Santa Marta (SNSM) in northern Colombia has recently proved an exception with no trace of Bd in recent mountain-range-wide screening (>2000 samples). Since the SNSM is of great importance for amphibian conservation due to its extreme diversity of phylogenetically distinct endemics, we investigated potential causes for this enigmatic absence and aim to predict disease susceptibility for the amphibian community to prepare conservationists for the pathogen's arrival. Besides extensive screening with qPCR and CRISPR diagnostics, we focused on modelling the biogeography of Bd spread, suggesting that lowland climatic barriers may have prevented Bd invasion thus far. However, as anthropogenic jump dispersal is an expected scenario, the arrival of Bd is likely only a matter of time. To rule out the possibility of historical presence and subsequent disappearance of Bd in the SNSM, we assessed population genomic structure – particularly potential bottlenecks in recent decades – in an endemic harlequin toad. We further conducted laboratory challenge assays using skin compound samples of three SNSM anuran species to test for potential skin defences inhibiting Bd growth. Ultimately, we conducted in vivo infection experiments of SNSM endemic anurans and caudates to

assess direct susceptibility. Our findings suggest high conservation concerns for safeguarding the Neotropics' last Bd-naïve amphibian community, translating into a need for urgent actions to implement biosecurity, conservation breeding and further research.

DISEASES

Exposure to the herbicide 2,4-D increases virulence of a global amphibian pathogen

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Abstract

Exposure to multiple environmental stressors threatens amphibian survival globally. While infections and pesticides are capable of driving declines, interactions may exacerbate their impact. Here, we study experimental exposure of painted frogs (*Discoglossus pictus*) to a commonly used herbicide (2,4-D) and/or to a presumed highly (JEL423) or low (BdBE3) virulent strain of the global panzootic lineage of *Batrachochytrium dendrobatidis* (BdGPL), a pathogenic fungus involved in amphibian declines and extinctions. Inoculation of both tadpoles and metamorphs with JEL423 impaired growth and reduced body condition of metamorphs, while inoculation with BdBE3 resulted in higher level infections that only reduced metamorph growth when inoculated at the metamorph stage. A higher pathogen burden at a lower cost for the anuran host corroborates the hypothesis of low virulent endemic BdGPL strains (BdBE3) versus highly virulent exotic strains (JEL423). Exposure of tadpoles to 2,4-D shortly before metamorphosis reduced froglet size at metamorphosis in a dose-dependent manner. Co-exposure to both 2,4-D during the tadpole stage and the BdGPL strains resulted in significant loss of body condition in the low virulent BdBE3 inoculated frogs, bringing its negative impact on par with that observed after inoculation with the highly virulent JEL423 strain. We demonstrate how the use of herbicides may exacerbate the impact of endemic infections on early life stage amphibians.

DISEASES

Current state of chytrid screening in natural, captive and archival amphibian populations from Poland

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Abstract

The first confirmation of *Batrachochytrium dendrobatidis* (Bd) infecting amphibians in Poland came from country-wide but sparse screening over the period 2014–2019 and indicated a prevalence of 122 out of 1506 (8.1%) in 9 species. Here we used quantitative PCR to screen archival alcohol-preserved individuals, and contemporary wild-caught and captive amphibians for chytrid fungi. We found that 93 out of 733 archival amphibians from 25 out of 80 sites were Bd-positive; the oldest archival samples dated Bd presence back to 1982. Numerous Bd-positive samples were found in the 1980's, 1990's and the first decade of the XXI century, with a wide geographical spread. We also swabbed 255 captive amphibians (29 anurans, 16 urodeles and 1 caecilian) in 2023-2024 and detected Bd in two collections of *Ambystoma mexicanum*; *Batrachochytrium salamandrivorans* (Bsal) was not detected. During the same period, we screened amphibian populations in one urban and one natural region to obtain estimates of prevalence at the landscape scale. In the urban area of Kraków, we found that 20 out of 302 amphibians (6.6%) from 4 out of 5 sites were Bd-positive. In the heavily-forested region of Magura National Park, we found that 176 out of 1411 amphibians (12.5%), from 83 out of 130 sites were Bd-positive. Most of the infections were found in *Bombina bombina* (Kraków), *B. variegata* and *Ichthyosaura alpestris* (Magura NP). In 2023-2024, we did not detect Bsal in any wild-caught individuals, despite checking numerous newt (*Lissotriton vulgaris*, *L. montandoni*, *I. alpestris*, *Triturus cristatus*) and salamander (*Salamandra salamandra*) populations. Based on our screening of archival, contemporary and captive amphibians, we conclude that Bd has been present and widespread in the country for at least the last three decades. Further monitoring is required to assess the threat of Bsal and to mitigate potential amphibian population declines.

DISEASES

The Overlooked Role of Non-Native Species: A Global Review of Pathogen Transmission in Freshwater Turtles

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Abstract

Freshwater turtles represent a particularly relevant group due to their popularity in the exotic pet trade and their frequent release into the environment. These releases, whether deliberate or accidental, raise concerns about the role of freshwater turtles as potential reservoirs or vectors of infectious pathogens. However, their contribution to pathogen transmission remains significantly understudied. To address this knowledge gap, we conducted a comprehensive review of the scientific literature on bacterial, viral, and fungal pathogens reported in freshwater turtles. Our analysis included 163 peer-reviewed articles, resulting in a dataset comprising 815 pathogen occurrence records across 188 pathogen genera. The most frequently reported host species were *Trachemys scripta*, *Pelodiscus sinensis*, and *Emys orbicularis*. Notably, *T. scripta*, an invasive species in many regions, was associated with a high number of zoonotic pathogens, including multiple strains of *Salmonella*, underscoring its importance as a disease vector. Our findings also revealed frequent pathogen sharing between native and non-native turtle species, with some distantly related taxa harboring similar pathogens, suggesting the influence of shared environments or anthropogenic factors. Bacterial pathogens were the most commonly recorded group, while viral pathogens revealed geographic links between Asia and North America. Fungal pathogens appeared to be more geographically restricted. The clinical effects on turtle health

were rarely documented in detail. Furthermore, non-native species remain underrepresented in disease surveillance and are often overlooked in One Health approaches. These findings highlight the urgent need to integrate principles of invasion biology into public health, disease ecology, and conservation. Recognizing non-native freshwater turtles as key players in pathogen transmission has important implications not only for wildlife health but also for broader One Health initiatives.

DISEASES

Dynamics and phenology of *Lucilia*-induced myiasis in common toads (*Bufo bufo*) inhabiting Southwest Germany

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Abstract

Lucilia bufonivora is a widespread amphibian-infecting parasitoid blowfly inducing primary myiasis. The almost always lethal myiasis apparently forces the amphibian host to switch from a nocturnal to a diurnal lifestyle. Although the phenomenon is known from across central Europe, there are few systematic records and analyses of myiasis on a more fine-scaled area. Since 2017 infected common toads (*Bufo bufo*) were collected in the "Harthaeuser Wald" (a forest patch in Southwestern Germany located east of Heilbronn) to collect phenological and morphometric data for Southwest Germany (Baden-Wuerttemberg).

Here we summarize results of the past six years. Myiasis was recorded from 400+ common toads, affecting all life history stages, including some metamorphosed toadlets. Myiasis started in late May and the last records were made around the onset of autumn in early October. Generally, female common toads were more frequently infested than males. However, infected toads maintained the typical sexual dimorphism regarding body size and mass. It seems plausible that the preference for female common toads is related to larger body size.

DISEASES

Ranavirus and Bd coinfection in Pyrenean mountain lakes change amphibian skin microbiome communities

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Abstract

Two emerging infectious diseases, chytridiomycosis and ranavirosis, pose serious threats to amphibian diversity. As the amphibian skin microbiome has been identified as major component of host immunity, a better understanding of the interaction between pathogens and the skin microbiome could help disease mitigation. For the first time, we investigated the presence of ranavirus in a large range of the French Pyrenees over 2008-2022, 51 localities and 2,622 individuals. In a subsample of 283 tadpoles of *Rana temporaria* and *Alytes obstetricans*, we further collected skin microbiome to investigate the adaptive microbiome hypothesis with ranavirus and co-infection with *Batrachochytrium dendrobatidis* (Bd). Although animals were asymptomatic of ranaviral infection, the presence of the virus was found in a large proportion of sampled localities and years, generally with low prevalence and loads. Our results generally validate the adaptive microbiome hypothesis in both amphibian hosts, as the virus altered the overall composition of the microbiome (beta-diversity). The alteration translated in a marked increase in putative antiviral genera in *R. temporaria*. However, the way in which the pathogen affected microbiome alpha-diversity varied from one host species to another. While both Bd and ranavirus had a significant impact on the amphibian skin microbiome composition, Bd was a more important predictor than ranavirus in both hosts, maybe linked to the higher destructive power of Bd in our populations. Our findings suggest that the amphibian skin microbiome could finely adapt to the deleterious presence of one or more pathogens, playing a key role in disease mitigation.

DISEASES

Susceptibility of the amphibian pathogen *Batrachochytrium dendrobatidis* to toad toxins

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Abstract

Chytridiomycosis is a widespread amphibian disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd). The severity of epizootics varies among regions, possibly due to differences in the virulence of local Bd lineages or in the susceptibility of host populations. Bufonid toads produce steroidal bufadienolide compounds, which may also be effective against Bd. Major differences have been found in toxin production among toad populations; however, it is unknown whether these differences translate into varying effectiveness against Bd, and whether the toxin profiles typical for different toad populations are more effective against syntopic or allotypic Bd strains. To address this knowledge gap, we performed in vitro growth inhibition experiments to characterise the growth inhibitory activity of toxin mixes collected from European toads (*Bufo bufo* and *B. spinosus*) in five European countries on Bd isolates obtained at the same localities. We quantified the bufadienolide compounds present in the toxin mixes using HPLC-MS, then treated all liquid Bd cultures with the serial dilution of each country's toxin mix and measured the optical density of the cultures after one week of incubation. Our results show that bufadienolide toxins can inhibit Bd growth and that the inhibitory efficiency depends on the origin of both the toxin mix and the Bd isolate. A syntopic or allotypic origin of the toxin mixes and the Bd isolates did not systematically influence the observed growth inhibition. The toxin mix collected in the UK was the most potent and the mix collected in Hungary was the least potent against Bd isolates. The Bd isolate obtained in the UK was the most resistant and the Spanish isolate the most susceptible to toad toxins. Future work should identify which individual bufadienolide compounds are responsible for effective inhibition, and whether toxin mixes that are more effective against Bd are also more effective against other pathogens or predators.

Poster presentations



CONSERVATION

Population genetics and genomics of fire salamanders (*Salamandra salamandra*) in Saxony (Germany)

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Abstract

Monitoring and conservation programs often focus on rare species, while comparatively common species tend to be systematically under-recorded. In Germany, one such species is the fire salamander (*Salamandra salamandra*), which is considered moderately common, yet most populations are rather cryptic. To alleviate this knowledge gap for eastern Germany, a monitoring project was carried out in the state of Saxony in 2024. The northeastern range limit of the fire salamander runs through this area, making the populations particularly interesting for studying isolation

effects and gene flow in regards to population genetics and the potential to escape pathogen spreading. This region is also of particular interest for the long-term conservation of the species, as there has been no evidence of the chytrid fungus *Batrachochytrium salamandrivorans* (Bsal), so far.

We used the Schiemenz catalog - a collection of herpetological information in the former GDR - as a basis for a comprehensive mapping of fire salamanders in connection with a citizen science campaign and larval monitoring. Additional populations in Saxony were selected for genetic analysis. The aim was to compare population structure and genetic diversity between large, connected populations and smaller, isolated ones. A combination of microsatellite analysis and double-digest restriction-site associated DNA sequencing (ddRADseq) was used to estimate indices of genetic diversity and infer levels of gene flow. Preliminary results of the analyses show surprisingly low genetic differentiation among *S. salamandra* populations in Saxony, despite geographical barriers suggesting limited gene flow. Yet, genetic diversity remains relatively high even in areas with a limited number of confirmed individuals. These findings suggest that fire salamanders can maintain high genetic diversity and adaptive potential even in small and geographically isolated (sub)populations, providing confidence regarding future conservation actions.

OTHER

Artificial light at night and influence on thyroid gland histology in *Triturus newts* (Urodela, Salamandridae)

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Abstract

Artificial light at night (ALAN) is one of the major causative agents of global sky brightening during night and drastically increased with urbanization, socio-economic development and transportation. During the past decade used of Light Emitting Diodes (LEDs) technology is increased. Beside many benefits to humankind allowing fundamental activities light during night have potential negative impact on human health and wild life representing one of the main contributors to global change. ALAN have great impact on amphibians, the most threatened vertebrate group globally. This study aimed to investigate the effects of constant nighttime light exposure on the thyroid gland of metamorphosed juvenile *Triturus ivanbureschi* through histological analysis. We utilized LED lighting as the primary outdoor light source, choosing an intensity of 30 lux and a cool color temperature of 6000K. The juveniles were randomly assigned to two experimental groups: a control group exposed to natural dark at night (<0.1 lux), and an experimental group exposed to 30 lux, 6000K LED light during the night for two months. During day both groups were exposed to 6000K LED lights intensity of 450 lux to imitate natural day light conditions. Thyroid glands were examined using standard histological techniques (H&E). Thyroid glands in the control group appeared more uniform, with smaller follicles, squamous to low cuboidal epithelium, filled with homogenous, dense colloid in the lumen. In contrast, glands from the light-exposed group exhibited larger follicles with taller epithelium, ranging from cuboidal to columnar with some follicles show scalloped colloid in the lumen indicating on

increased thyroid activity or hyperfunctioning. This pilot study was conducted on a small number of animals, and further research is needed to explore effects during the larval stage and under different lighting conditions.

CONSERVATION

Ecological niche modeling reveals future threats to *Ceratophrys aurita* (Anura: Ceratophryidae), a species with already missing populations in the Atlantic Forest

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Abstract

Amphibians are the most threatened vertebrate group worldwide, with several species undergoing marked population declines. In Brazil, *Ceratophrys aurita* is a rare frog species predominantly associated with the Atlantic Forest, currently listed as Least Concern (LC), despite indications of substantial distributional contraction. A recent historical review revealed that this species has not been recorded in over 70% of its historical localities since 1990, suggesting a loss of 71.9% in area of occupancy and 28.3% in extent of occurrence. To assess future risks, we compiled 273 occurrence records and developed ecological niche models under current and future climate and land-use scenarios (SSP2-4.5 and SSP5-8.5 for 2050 and 2070). We used bioclimatic variables from WorldClim 2.1 and selected the most relevant predictors based on Variance Inflation Factor (VIF) analysis. Models were calibrated using eight algorithms in the biomod2 R package and validated with True Skill Statistic (TSS) and Sørensen Similarity Index metrics. Temperature seasonality (BIO4) was the most influential variable, contributing 42.6% to model predictions, followed by mean diurnal temperature range (BIO2, 14.2%) and mean temperature of the wettest quarter (BIO8, 12.3%). Projections indicate a north-to-south contraction in habitat suitability, particularly under extreme scenarios. To estimate habitat loss, we combined model outputs with land-use change projections, applying species-specific land cover masks based on the IUCN habitat classification scheme. The results indicate a projected loss of suitable habitat of 75.65% under SSP2-4.5 and 74.69% under SSP5-8.5. These findings reinforce concerns raised by historical data: *C. aurita* has already lost many populations and faces further risks from climate and land-use change. We highlight the urgent need to reassess its conservation status and to prioritize field surveys in both historically known and climatically suitable areas.

OTHER

The Global Women in Herpetology Project: The Journey So Far and What's Next

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Abstract

Gender equality remains a major issue globally. Though progress has been made, the number of women is lower than that of men at all career stages, and data shows that representation decreases as the career stage increases. Societal structures, cultures, norms, and traditions have been identified as factors contributing to gender inequality. However, exposure to diverse role models and broader representation within STEM have a positive effect on the recruitment and retention of students and early-career biologists, especially from underrepresented groups in STEM. To increase the visibility of women in herpetology across disciplines, regions, and cultural backgrounds, we formed the Global Women in Herpetology Project and gathered herpetologists from 50 countries and regions. Collectively, we published "Women in Herpetology: 50 Stories from Around the World," which contains short stories in which each herpetologist shares a personal narrative that intersects their gender, cultural background, and professional journey. We then used the profits of the book to establish a conference scholarship for female students in underrepresented regions around the world. Through this initiative, we hope to promote further opportunities for collaborations and mentorships for women in the field of herpetology and to engage more people to join our efforts. The next phase of this project is to widely translate the "Women in Herpetology" book into different languages, starting with Spanish and Chinese. Through this initiative, our aim is to collectively place a spotlight on the diversity of women in our field and encourage more young people to enter the field of herpetology.

EVOLUTION

Do pyrethroid insecticides influence sex ratios in agile frogs (*Rana dalmatina*)?

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Abstract

Environmental stressors, including extreme temperatures and chemical pollutants, can influence the sex ratio of wildlife populations by two main mechanisms: sex-biased mortality and disrupted sex determination. The latter includes sex reversal, whereby young individuals develop the phenotypic sex opposite to their genotypic sex. Skewed sex ratio has wide-ranging evolutionary implications for reproductive systems, social behaviors, population adaptability, and extinction risk. Our study investigated the

effects of the pyrethroids deltamethrin and etofenprox on the sex ratio of agile frogs (*Rana dalmatina*). These chemicals are commonly used as insecticides, including mosquito control and agricultural applications in wetlands, yet their endocrine disruptor effects are very little explored in amphibians. In our study, we exposed tadpoles, derived from field-collected eggs to environmentally relevant concentrations (0.03 or 0.3 $\mu\text{g/l}$) of deltamethrin or etofenprox in outdoor mesocosms. The water was treated three times during the rearing period: on the 1st, 15th, and 35th days of the experiment. Two months after metamorphosis, we determined the phenotypic sex of the frogs by dissection and identified the genotypic sex using molecular markers. We analyzed whether any treatment increased the frequency of sex reversal and whether mortality during metamorphosis depended on genotypic sex in any treatment group. We found that the lower concentration of etofenprox shifted the phenotypic sex ratio towards males, however, this shift was due to female-biased mortality rather than sex reversal. This finding emphasizes the importance of distinguishing between sex-specific mortality and sex reversal when evaluating the impacts of endocrine disruptors on sex ratios and for predicting and mitigating their long-term evolutionary impacts on biodiversity.

CONSERVATION

Herpetofauna along an urbanization gradient: case study from a city with ongoing industrialisation

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Abstract

Habitat loss and degradation is a worldwide threat to amphibian and reptile populations. Increasing urbanization is among the major causes, and climate change may worsen the scenarios. Debrecen, the second largest Hungarian city is located in the Great Hungarian Plain, at the meeting point of saline and sandy grasslands and wetlands, which historically provided habitat for rich and abundant herpetofauna. However, the city now experiences intensive urbanisation and large-scale industrial developments, and climate change strikes the region with unusually hot temperatures and droughts. Therefore, the University of Debrecen started a comprehensive biomonitoring programme to follow up and potentially mitigate the effects of these large-scale changes.

In this research first we mapped the herpetofauna in 54 sampling sites in scattered in urban, suburban and natural areas within and around Debrecen. Species presence was collected by visual and acoustic observations along transects by visiting each study site multiple times. We detected altogether 12 species in the whole sampling area (7 in the most species-rich sampling point), while the *Pelophylax* complex and *Lacerta agilis* were the most abundant. We analysed the effect of urbanisations and aquatic habitats on species richness in each site by linear models. So far, the results indicate that species richness is negatively affected by the urbanisation gradient, but increases in the proximity of aquatic habitats.

This research clearly demonstrates the negative impacts of urbanization and climate change on biodiversity. Follow-up monitoring of these sites will allow us to understand how ongoing climatic and environmental changes affect species composition and richness, and hopefully will help raise public awareness and plan efficient conservation actions to preserve a diverse biota throughout the urbanisation gradient.

CONSERVATION

Conservation of three newt species in the core range of the microendemic Calabrian Alpine newt (*Ichthyosaura alpestris inexpectata*; Catena Costiera, Southern Italy): population dynamics, impacts of invasive fish, and habitat assessment

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Abstract

The introduction of non-native fish is a major cause of amphibian decline, especially in newts, highlighting the need for habitat restoration efforts. We surveyed *Triturus carnifex*, *Lissotriton italicus*, and *Ichthyosaura alpestris inexpectata* across nine originally fishless lentic habitats in southern Italy, encompassing both fish-free and fish-invaded wetlands. These sites, representing the core range of the micro-endemic *I. a. inexpectata*, include both temporary and permanent systems. One pristine, fishless lake occurs in the Special Area of Conservation (SAC) "Laghicello", while the other eight sites are in or near the SAC "Laghi di Fagnano", which includes 3 sites with introduced fish. From March to July 2025, we conducted 1 to 3 monthly surveys by dip nets and glow stick-baited funnel traps. Newts were individually photo-identified using their distinctive coloration patterns to enable capture-mark-recapture population estimates. Biometrics and epithelial samples were also collected for screening for *Batrachochytrium dendrobatidis* (Bd). Differences emerged in newt abundance between fish-free and invaded sites, and between temporary and permanent ponds. At Laghicello, we found 711 *T. carnifex*, 405 *I. a. inexpectata* and 59 *L. italicus*. Across the eight other sites, we captured 382 *I. a. inexpectata*, 341 *T. carnifex*, and 267 *L. italicus*,

with recapture rates and abundance varying by taxa and site. We found 79 paedomorphic *I. a. inexpectata* (7 males, 69 females) at Laghicello (11.4% of captures) and three fish-invaded sites (frequency: 9 - 29%), indicating complex responses to fish presence. Preliminary findings suggest an association between fish presence, newt abundance, and shifts in habitat use. As part of a broader conservation and restoration project, ongoing analyses will provide data on species-specific population dynamics, breeding success, and Bd prevalence, while assessing the outcomes of removal efforts and fish impacts on unmanaged sites.

ECOLOGY

Built to Hunt, Forced to Scavenge: Scavenging behaviour in an insular population of the nose-horned viper (*Vipera ammodytes*)

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Abstract

Insular populations often face ecological challenges distinct from their mainland counterparts, particularly due to limited resource availability. These constraints can lead to notable, morphological, physiological and/or behavioral adaptations, such as stunted growth (dwarfism), modified diet and altered foraging strategies. The isolated population of the Nose-horned vipers (*Vipera ammodytes*) on Golem Grad Island exhibits such adaptations, including restricted prey base, consisting mainly of lizards and centipedes. The specific diet is likely one of the main drivers of reduced body size and fecundity observed in the population. In this study, we document three instances of scavenging behavior by these vipers on avian carrion – an uncommon feeding strategy in this species. Two events were observed during nighttime, involving the consumption of a dead alpine swift (*Tachymarptis melba*) and a dead European goldfinch (*Carduelis carduelis*). The last observation was recorded during the day, with a viper feeding on an unidentified dead passerine bird. These observations suggest that, under ecological pressures such as food scarcity, insular *V. ammodytes* may broaden their dietary niche to include opportunistic scavenging, a behavior not commonly associated with vipers. This finding highlights the behavioral flexibility of insular snake populations under resource-limited conditions.

ECOLOGY

Substrate preference and its effects on body temperature in a rock-dwelling nocturnal gecko, *Hemidactylus turcicus*, on an Aegean Island (Bozcaada, Türkiye)**Begüm Boran**

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Abstract

Thermal ecology and substrate use of nocturnal geckos remain understudied, despite their reliance on behavioral thermoregulation in the absence of solar radiation. This study investigated nighttime substrate preferences and their thermal consequences in *Hemidactylus turcicus* (28♂, 20♀) on an Aegean Island (Bozcaada, Türkiye) during spring by assessing substrate type (shelter, crevice, open) and size (large, small). Additionally, body (T_b) and substrate temperatures (T_s) were measured to explore potential links between substrate preferences and degree of active thermoregulation ($\Delta T_s = |T_b - T_s|$). Rock crevices were the most frequently used substrate type by both sexes, suggesting their importance as nighttime refugia and sources of thermal stability. While males predominantly used small rocks, females did not display a distinct preference for any particular substrate size. T_b significantly varied with substrate type, with females showing differences between open and crevice substrates, while males exhibited differences between open vs. crevice and open vs. shelter substrates ($p < 0.05$). Both females ($p: 0.027$) and males ($p: 0.000$) exhibited significantly higher T_b values on large rocks compared to small ones. ΔT_s was significantly greater in females than in males ($p: 0.038$), implying sex-specific thermoregulatory strategies. A strong positive correlation was found between T_b and T_s ($r: 0.883$, $p < 0.001$), highlighting the critical role of rock temperature in influencing body temperature of individuals in their natural habitat. These results underscore how nocturnal ectotherms rely on substrate features to navigate thermal heterogeneity and sustain physiological function.

Acknowledgements: This study is part of the PhD thesis titled "Thermal Biology in Bozcaada Population of *Hemidactylus turcicus* (Linnaeus, 1758) (Sauria: Gekkonidae)", supported by the Çanakkale Onsekiz Mart University The Scientific Research Coordination Unit, Project number: FDK-2025-5073.

ECOLOGY

Tiny Colonizers of Frog Skin: Composition and Diversity of Diatoms in the *Pelophylax esculentus* complex

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Abstract

Diatoms, microscopic algae found in various aquatic environments, have recently been recognised as epibionts on the skin of amphibians, although their diversity and ecological role remain underexplored. In this study, the diatom communities on the skin of frogs of the *Pelophylax esculentus* complex are investigated at three freshwater sites in southern Banat (Serbia), which differ in the degree of eutrophication and anthropogenic pressure. A total of 59 frogs representing all three taxa of the complex were collected and sampled using standard algological methods: adhesive tape and brush scraping. A total of 203 diatom taxa from 54 genera were identified. Statistical analyses showed that the environmental conditions significantly influenced the structure of the diatom community. A two-way ANOSIM revealed significant differences between localities ($R = 0.23$, $p = 0.03$), but not between frog taxa ($R = 0.08$, $p = 0.24$). PERMANOVA confirmed a significant effect of locality ($F = 1.39$, $p = 0.03$), with no significant effect of frog taxa ($F = 12.35$, $p = 0.11$) or their interaction ($F = 10.64$, $p = 0.32$). Principal coordinate analysis revealed two environmental gradients: The first separated sites according to trophic state (mesotrophic, eutrophic, hypertrophic), with diatom diversity higher in eutrophic conditions; the second reflected saprobial levels and distinguished oligosaprobic/ β -mesosaprobic from α -mesosaprobic/polysaprobic habitats. The weak differences in the diatom communities between the frog taxa indicate that the environmental conditions and not the host taxa characterise these communities. This emphasises the ecological role of amphibian skin as a suitable microhabitat for periphytic algae and highlights the need for further research to determine whether it serves as a transient or stable habitat for diatom colonisation.

MORPHOLOGY

Morphological variability of the digestive system and dietary specificity in anurans

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Abstract

The general anatomy of the digestive system in vertebrates is quite conserved. However, different groups exhibit a marked variation mainly related to dietary specializations, such as ruminant and myrmecophagous mammals. Although anuran amphibians in their adult stage are considered generalists, some groups show specializations like myrmecophagy and frugivory. Despite this variability, few studies explore the morphological features associated with diet at the adult stage. Here, we analyze the morphological diversity of the digestive system in tree frogs (Hylidae: Hylinae) in which most of the dietary specializations described in amphibians are represented, (i.e., myrmecophagy, frugivory, and generalist diet). Anatomical studies were performed using iodine contrast-enhanced computed microtomography. The images were segmented and analyzed using Dragonfly and Slicer 3D programs, and were complemented with stereomicroscope dissections to study the myology of the tongue. Different morphological variables of the organs of the digestive system and the tongue were characterized and corrected for total body length and body cavity volume for comparisons using principal component analysis (PCA). Our results show noticeable differences between *Xenohyla truncata* (frugivorous) and the rest of the species, particularly in its considerably shorter small intestine. In contrast, *Phyllodites luteolus* (myrmecophagous) has a longer small intestine. The tongue musculature also revealed interspecific variability, specifically, in *X. truncata* possessing the shortest tongue. The marked divergence of this species from the rest of the clade could represent a particular evolutionary trajectory linked to an uncommon trophic niche among anurans. Overall, our findings provide evidence that, even within a group traditionally considered generalist, there are morphological traits that suggest adaptations to different feeding and ecological strategies.

ECOLOGY

Tracking Frogs Through the Concrete Jungle: Microhabitat Associations of Eight Anuran Species in Delhi-NCR

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Abstract

Understanding fine-scale microhabitat preferences and activity patterns is critical for amphibian conservation, especially in fragmented and urbanized ecosystems like Delhi-NCR. This study documents the microhabitat associations and seasonal activity of all eight known anuran species from the region: *Duttaphrynus melanostictus*, *D. stomaticus*, *Euphlyctis cyanophlyctis*, *Hoplobatrachus tigerinus*, *Minervarya pierrei*, *Microhyla nilphamariensis*, *Uperodon systoma*, and *Sphaerotheca maskeyi*. *D. melanostictus* is active during western disturbances (February) and post pre-monsoon showers (May), inhabiting urban microhabitats like small ephemeral water bodies and roadside drains. *D. stomaticus* is active mainly in July–August, calling from shallow, rain-filled pools in moderately disturbed urban areas. *E. cyanophlyctis* is active year-round and commonly found in permanent urban water bodies such as ponds, lakes, and cemented reservoirs, often observed near the edges or partially submerged. *H. tigerinus* emerges during the pre-monsoon season (May), preferring open, sunlit temporary pools formed after the first rains. *M. pierrei* is active from March through the monsoon, found in shaded patches near ephemeral puddles in parks and forest edges. *M. nilphamariensis*, active during the monsoon, uses damp, shaded crevices and shallow ground depressions that retain transient water near wet zones. *U. systoma* is fossorial and surfaces for about 24 hours during intense monsoon rains, breeding in open ephemeral pools with loose, moist soil ideal for burrowing. *S. maskeyi* is surface-active for 2–3 weeks during peak monsoon and prefers partly shaded ephemeral pools with soft soil in urban green spaces. This study provides a baseline of species-specific microhabitat preferences and activity timing, highlighting

the importance of conserving small, overlooked habitat patches essential for amphibian persistence in rapidly urbanizing landscapes.

EVOLUTION

Mitochondrial DNA suggests a narrower hybrid zone in southern than in northern Poland in *Hyla* treefrogs

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Abstract

Hybrid zones serve as natural laboratories for studying speciation and gene flow. We investigated the mitochondrial structure of the *Hyla arborea* × *H. orientalis* hybrid zone in southern Poland using 16S rRNA data from 421 individuals, combining new sampling with previously published data. Divergence in the 16S rRNA gene between the two species amounts to 3.0%. Mitotype assignments revealed a narrow transition zone approximately 45 km wide (extending to ~100 km with an isolated outlier), contrasting with the much broader cline (~200 km) described in northern Poland, and comparable in width to the steep mitochondrial transition observed in the Balkans (~30 km). The steep mitochondrial cline in southern Poland suggests limited introgression and possibly stronger reproductive isolation, or an older contact zone. Differences in climate stability, population history, or time since secondary contact may underlie this disparity, but mitochondrial data alone cannot fully resolve hybrid zone dynamics. Given known cases of mitonuclear discordance in treefrogs, nuclear genomic data are essential to assess the extent of hybridization, the directionality of gene flow, and potential reproductive barriers shaping this zone. Further genomic work will be needed to determine whether the nuclear genome follows the steep mitochondrial cline.

CONSERVATION

Conservation of the Calabrian Alpine Newt: A Pilot Reinforcement Project in Southern Italy

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Abstract

The Calabrian Alpine newt (*Ichthyosaura alpestris inexpectata*), an endemic amphibian of southern Italy, is increasingly threatened by habitat loss and the introduction of alien fish. Alongside efforts such as invasive fish removal and habitat restoration, establishing a captive-bred reservoir population for population reinforcement offers a complementary strategy to prevent further decline. In 2023, 40 individuals (20 founder pairs) were collected and transferred to the Aquatis Aquarium-Vivarium in Lausanne (Switzerland), where refined husbandry protocols led to successful reproduction. After pathogen screening, 595 captive-reared individuals (515 juveniles photo-marked using their distinct lateral spot patterns and 80 larvae) were released into native sites in Calabria in November 2024. Georeferenced release sites were selected based on shelter availability, habitat quality, and minimal human disturbance. Larvae were acclimatised in semi-submerged in situ cages for 40 days before release, during which 66% metamorphosed successfully. Weekly post-release monitoring was followed by active search during the first month, with a broader standardised monitoring program started in February 2025 (dip nets and aquatic funnel traps) across the entire breeding season. Photo-marking of captive-bred individuals, combined with capture-mark-recapture, will enable the tracking of survival and dispersal, the identification of key breeding sites, potential ecological barriers, and habitat corridors, as well as the impact of threats, on repopulation success and natural recruitment. This approach aims to improve future release protocols and monitoring, strengthening long-term conservation of *I. a. inexpectata*. The obtained data will guide ongoing conservation efforts, such as fish removal and the creation of satellite ponds, and contribute to the development of reliable demographic models that are helpful in estimating population trends and assessing extinction risk.

CONSERVATION

Amphibian PhotoID: Hotspotter stands out!

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Abstract

Individual monitoring is a major part of conservation biology, especially to study population dynamics and detect potential declines. Amongst the available methods, photo-identification (photoID) sets itself apart by being non-invasive and having a low cost. Although visual recognition can be enough at a small scale, a high number of individuals quickly makes the use of software essential.

In this study, the performances of four widely-used photoID programs were evaluated for 11 species of european amphibians. Our results show that Hotspotter stands out with recognition rates close to 100 % for most tested species. Only the fire salamander (*Salamandra salamandra*) had a lower rate (70 %).

The standardisation of pictures was also shown to improve performances for all tested programs.

Our results outline Hotspotter as the best performing software for the individual monitoring of european amphibians, even in multi-species contexts. Making these results accessible is essential to encourage less-invasive monitoring studies, which will also be more accessible for structures with less funding.

ECOLOGY

Preferred body temperature of the Caspian whipsnake (*Dolichophis caspius*, Gmelin, 1789) under laboratory conditions

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Abstract

In ectothermic animals, such as snakes, the body temperature depends on the temperature of the external environment. However, maintaining optimal body temperatures is crucial for various physiological functions such as feeding, locomotion, reproduction and immune response. In addition to interspecific variability, snake body temperatures can also vary at the intraspecific level (between populations, sexes and age classes) and are influenced by factors such as body size, presence of food in the stomach, gravidity, shedding phase and season. Although thermal biology is important for understanding organismal biology and conservation, it remains understudied in many common species. This study aimed to analyse the thermobiological traits of the Caspian whipsnake (*Dolichophis caspius* Gmelin 1789) under laboratory conditions, considering the effects of sex, age and season. We measured the cloacal temperatures of 15 adults (8 males and 7 females) and 14 juveniles (7 males and 7 females) hourly, from 09 to 18h. The snakes were exposed to a thermal gradient (17-45°C) in terraria. The influence of sex and age on the preferred body temperature (T_b) of individuals in the thermal gradient was modelled using general linear mixed models. We found that T_b did not differ between age groups ($p=0.118$) and sexes ($p=0.238$), but there was a statistically significant interaction ($p=0.037$). The mean value of the preferred body temperatures for all individuals was 24.5 ± 4.9 °C (range 13.3-39.6°C). Set-point range (T_{set}) that represents the central 50% of all body temperatures selected in the thermal gradient, varied between 15.8°C and 31.9°C, with a mean of 24.3 ± 3.5 °C. The difference in T_b between seasons was only tested in juveniles and the results showed statistically significant effects of sex ($p<0.001$), season ($p<0.001$) and their interactions ($p<0.001$), with lower T_b in spring than in summer, and males having higher T_b than females in spring and lower in summer.

CONSERVATION

Impact of urbanisation on local amphibian diversity: an example from Belgrade city and suburbia

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Abstract

Urbanisation fragments habitats, increases isolation, hinders amphibian migration and causes population decline. Urban pollution significantly impacts amphibian physiology, behaviour, ecology and health. Tolerant species dominate, as sensitive ones often cope poorly with environmental change. We surveyed 13 Belgrade urban sites (2022–2023) - an area of 1720 ha - for amphibians and found 611 individuals from 7 species. Site attributes, including the 500 m surrounding area, were analysed in QGIS. Attributes were correlated using Kendall Tau, excluding but one if $p \geq 0.9$. Observed individual numbers were correlated with site attributes per species using Kendall Tau, applying 20% FDR and LOOCV for stability. Due to the small sample size, only strong, stable correlations ($p \geq 0.5$; LOOCV ≥ 0.7) are reported. All lengths are in km, all areas in ha. For *Rana dalmatina* presence, a strong correlation was found with the number of lakes at a site ($p = 1.00$; LOOCV = 92.31%). Green frogs presence correlated with: Urban environment area around site ($p = -0.70$); roads length around site ($p = -0.67$); distance to nearest natural environment ($p = -0.63$); distance to nearest river ($p = -0.58$); footpaths quantity at site ($p = -0.53$); rivers length around site ($p = 0.51$); running water length around site ($p = 0.51$); running waters number around site ($p = 0.53$); streams length at site ($p = 0.54$); rivers number at site ($p = 0.54$); running water length at site ($p = 0.55$); green areas size around site ($p = 0.58$); rivers number around site ($p = 0.59$); standing water area at site ($p = 0.62$); streams number at site ($p = 0.64$); Running water number at site ($p = 0.73$) – all LOOCV = 100%. No significant results for *Bombina variegata*, *Bufo bufo*, *Lissotriton vulgaris* and *Salamandra salamandra*.

DISEASES

CMTV-like ranavirus detection during a mass die-off of common frogs in a high-altitude alpine lake in Italy

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Abstract

Alpine lakes represent fragile ecosystems vulnerable to anthropogenic pressures and environmental changes, which may facilitate the emergence of infectious diseases among native amphibians. In August 2024, we investigated a mass mortality event affecting a population of common frogs (*Rana temporaria*) at Lago Lungo (2503 m a.s.l.) in a high-altitude alpine lake in Piemonte, Italy, where 78 dead adult frogs were recorded. Nine minimally decomposed carcasses underwent pathological examination, bacteriological culture, and molecular diagnostics. Clinically, frogs exhibited hyperaemia, particularly at the base of the hind thighs. Molecular analysis excluded chytrid fungus (*Batrachochytrium dendrobatidis*) and herpesvirus infections but detected ranavirus DNA in skin samples from four individuals. Sequencing of two samples identified a common midwife toad virus (CMTV)-like strain, closely related to isolates previously reported from ranavirus outbreaks in the French Alps. Opportunistic bacterial pathogens (*Hafnia alvei*, *Acinetobacter guillouiae*, *Acinetobacter proteolyticus*, *Serratia proteamaculans*) were also isolated from vitreous humour, suggesting potential secondary bacterial infections. The detection of a CMTV-like ranavirus strain highlights a serious health threat to alpine amphibian populations, especially in areas already subject to ecological stressors such as climate warming,

livestock grazing, and introduced fish. Our findings represent the first confirmation of ranavirus-associated mortality in *R. temporaria* in Italy, reinforcing the need for enhanced surveillance and proactive pathogen screening in alpine amphibian conservation strategies.

CONSERVATION

Wildfire-Induced Mortality and Population Decline of *Testudo marginata* in Two Mediterranean Habitats**Maria Dimaki**

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ECOSTUDIES P.C.

Abstract

Since 2000, the extent of burned areas in Greece has steadily increased, primarily due to a rising frequency of wildfires. Populations of the Marginated Tortoise (*Testudo marginata*) have been significantly affected, particularly in southern Greece. This study assessed the impact of wildfires on *T. marginata* populations in two areas with known population sizes in the Attica region.

Using capture–recapture methods, population density was estimated at 3.52 individuals/ha in a 41.3 ha area on southern Mount Imittos in spring 2021. At a second site in northern Lavreotiki, within an 8 ha area, density was estimated at 2.75 individuals/ha. In addition, eight individuals, at the Imittos, site were fitted with radio transmitters and were tracked for two years.

In August 2021, the Lavreotiki site was affected by a severe wildfire. Based on carcass counts, immediate post-fire mortality was estimated at a minimum of 50%. Capture–recapture data from spring 2023 indicated a total population decline of 77.3%. The Imittos site experienced a wildfire in summer 2022. Mortality among radio-tagged individuals was 25%, while carcass surveys suggested an overall mortality rate of at least 9.7%. Capture–recapture data from spring 2024 indicated a population decline of 21.4%.

The marked difference in mortality between the two areas is attributed primarily to differences in fire behavior. The Lavreotiki fire, characterized by low wind and high temperatures, resulted in complete vegetation loss. In contrast, the Imittos fire, driven by strong winds, spread rapidly and left patches of unburned vegetation. Additionally, certain microhabitat features on Imittos may have served as refugia, likely contributed to the lower tortoise mortality observed.

ECOLOGY

Three Decades of Chameleon Research in Greece: Insights into the Distribution, Ecology, and Conservation of *Chamaeleo chamaeleon* and *Chamaeleo africanus*

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ECOSTUDIES P.C.

Abstract

The African Chameleon is an allochthonous species for Greece and its presence in the area of Gialova Pylos (NATURA2000 site GR2550008) was documented by Prof. W. Böhme in 1989. The presence of the species in Peloponnese is likely due to its introduction in historical times. Based on morphological characters and mitochondrial 16S rRNA sequences, it is suggested that the Greek population of *C. africanus* originated from the Nile Delta region of Egypt. The recent years (2013) two new populations of the species were discovered in the western Peloponnese (GR2330005 and GR2320001 sites). These populations are due to the local translocation of the species by chameleon's enthusiasts. The mean population density of the species was estimated at 9.69 ind/ha, using the capture-marking-recapture and the line transect method.

In Greece, the known distribution of the Common Chameleon included the islands of Samos, Chios, and Crete. As observation from Chios island was never confirmed, and observations from Crete island are probably due to individual animals transported by humans, we conclude that the species is restricted to Samos island. A new reproductive population of the Common Chameleon was discovered in 2014, at the Northern suburbs of Athens, unknown if it was an accidental or intentional importation. The population density of the Common Chameleon was estimated at 5.26 ind/ha.

No statistically significant difference was found in the sex ratio for either chameleon species.

Both species are threatened by loss of habitat (through fires, agricultural intensification and development of tourist facilities), illegal collection and road mortality. Also, from predation mainly by snakes, rats, falcons, owls, weasels, cats and dogs, wild boars and feral pigs, also from foxes in Peloponnese. Both species are assessed as Endangered (EN) at the Greek Red Data Catalogue (2024).

OTHER

Mobilizing biodiversity data from the literature – presenting BIOfid

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Abstract

We are currently facing a global-scale biodiversity crisis, with accelerating rates of extinction, habitat degradation, and ecosystem disruption. Notwithstanding, the recent past of many organisms is poorly known, especially when the available information is fragmented and scattered in old texts. Published scientific literature is a valuable source of ecological data, but this source remains underexploited due to several

limitations, such as difficulties in accessing those texts, language barriers, and the fact that the data is not yet FAIR. Thus, such a powerful source of information is often neglected in research as extracting the information is rather time-consuming. Technological advances such as optical character recognition, natural language processing, and artificial intelligence can facilitate the acquisition of information hidden in text sources. The Specialised Information Service for Biodiversity Research (BIOfid; <https://www.biofid.de>) aims to increase availability, accessibility, and usability of contemporary and historic biodiversity information. The newly developed tool, the UCE (Unified Corpus Explorer), allows the access and mobilization of biodiversity data hidden in texts. By annotating elements relevant to biodiversity research, BIOfid aims to establish interoperability and reusability of data from literature, contributing for a better understanding of the past, present, and future of biodiversity. We currently focus on plants, birds and butterflies, their spatial occurrences, biotic interactions (e.g. insect host plants) and morphological traits.

MORPHOLOGY

Artificial light at night and influence on eye structure in *Triturus newts* (Urodela, Salamandridae)

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Abstract

Light pollution is one of the modern anthropogenic impacts on the environment that is growing rapidly. The disruption of the natural day-night regime imposed by artificial light at night (ALAN) places significant pressure on biological systems. So far it has been observed that ALAN causes disturbances in the metabolism, reproduction, and behavior of animals, but it is less known how it affects the visual system, especially in amphibians. Newts are mostly nocturnal animals with complex lifecycles and they may be sensitive to changes in the environment such as ALAN. This research aimed to examine how different intensities of long-term exposure to Light Emitting Diodes (LEDs) light during the night can affect retina of larvae, and metamorphosed juveniles of *Triturus* sp.

We used LED lights with intensities of 5 and 30 lux, which correspond to the actual intensities of ALAN in area inhabited by amphibians, and two light color temperatures: warm (2700K), and cold (6000K) light. After hatching and up to the 62 stage, larvae were divided into five experimental groups: the control group (artificial daylight + dark at night) and four treatment groups with LED lights: 5lux 2700K, 5lux 6000K, 30lux 2700K, and 30lux 6000K during night. The animals were kept under these condition for three months. We also used metamorphosed juveniles which were divided immediately after metamorphosis into two groups – control and treatment (30lux 6000K), kept under these conditions for two months. Eye tissue was studied using histological methods of H&E staining as well as immunofluorescence for visual pigment rhodopsin detection. Our preliminary results indicate a higher intensity of the

fluorescent signal for rhodopsin in individuals that were treated in comparison with the control group, especially in juveniles. Further data analysis should provide a clearer insight into how ALAN affects different layers in the retina especially quantity and distribution of rhodopsin in rod cells.

DISEASES

Sheltering the Serpent: Habitat Optimization and Disease Monitoring in Barred Grass Snake Populations

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Abstract

Native snake populations in Germany are in steep decline, with many species classified as “endangered” or “critically endangered.” The Barred Grass Snake (*Natrix helvetica*) is particularly threatened by habitat loss, urban expansion, invasive predators, and emerging diseases such as ophidiomycosis caused by the fungus *Ophidiomyces ophidiicola*. Since 2021, we have conducted systematic monitoring of two habitats located within Wuppertal by using artificial hiding places. Capture-recapture surveys from 2021-2024, compared against baseline data from 1986-1991, reveal significant declines – especially among adults of reproductive age – while the majority of the observation comprise juvenile snakes.

To counter these trends, we evaluated different refuge designs and identified a clear preference for dark microhabitats in previous research, a principle that we continue to use today to improve habitat design and conservation strategies. An increase in capture rates during 2024 suggests early success of these habitat-optimization measures. Ongoing efforts will focus on refining refuge placement.

One reason for the decline in findings could be related to the occurrence of *O. ophidiicola* in the area. The pathogen was first detected in the study area in 2024 and we currently found animals with a confirmed infection again.

Our results underline the need to protect the natural habitats of *N. helvetica* and to monitor the populations in the long term in order to detect threats such as pathogens at an early stage so that countermeasures can be taken.

ECOLOGY

Same mountains, different plastics? Lizards and microplastic exposure

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Abstract

Microplastics have become widespread pollutants in both terrestrial and aquatic environments. While their impacts have been extensively studied in marine ecosystems, data on microplastic exposure in terrestrial reptiles remain scarce. Recent studies have reported microplastic ingestion in various lizard species, primarily in the form of synthetic fibers from anthropogenic sources, likely ingested through trophic transfer or direct contact with contaminated substrates.

The Oukäïmeden region of the High Atlas Mountains in Morocco offers a valuable setting to explore this issue. This high-altitude area hosts various lizard species that coexist in close proximity and share overlapping habitats, including the gekkonid *Quedenfeldtia trachyblepharus* and the lacertids *Atlantolacerta andreanszkyi*, *Scelarcis perspicillata*, and *Podarcis vaucheri*. Although some interspecific differences in microhabitat use and diet have been reported, all four species are likely exposed to similar environmental sources of microplastics, with ingestion occurring via incidental consumption during foraging or through contaminated prey.

This study investigates the presence and characteristics of microplastics in the faeces of these four sympatric lizard species. Preliminary results indicate higher presence of coloured fibres, and different quantities of microplastics in the different species with

Atlantolacerta andreanszkyi having higher prevalence and quantity of microplastics. The work contributes to understanding the distribution of pollutants in high-altitude ecosystems and provides a foundation for future research on the ecological and physiological impacts of microplastic exposure in terrestrial reptiles.

CONSERVATION

Conservation of threatened populations of Agile Frog, (*Rana dalmatina* Bonaparte, 1838) in Italy: when the difference is not remaining indifferent

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Abstract

The data presented concern Mount Maddalena of Brescia (Lombardy) and Special Conservation Area ITA6030018 'Cerquone - Doganella' (Lazio), two important breeding areas of *Rana dalmatina* in Italy, a species listed as LC but declining by IUCN Red List, monitored for years (since 2014 and since 2010 respectively). Both Sites are recognized as Areas of National Herpetological importance (ITA133LOM033 and ITA056LAZ001) by Societas Herpetologica Italica.

The 4 ponds of Mount Maddalena underwent a fundamental renovation in 1993, with installation of PVC sheets to keep the water flowing from the slopes above, but since then the maintenance actions have only concerned aesthetic aspect of the area and safety of visitors (rebuilding the fence, cutting the perimeter vegetation). Progressively worsening water conditions (both due to the increasingly longer dry periods and the reduced volume of water for debris and aquatic vegetation) have determined progressive decline in *Rana dalmatina* reproduction, which has gone from 232 egg masses in 2016 to 32 in 2025. Doganella Wetland is located within a vast caldera depression and occupies an alluvial plain with an area of about 2.5-3 hectares. Water level varies seasonally, until it disappears during late spring and summer. The conservation program consisted of annual actions to save -during breeding season- migrating individuals through perimetally very busy roads, with installation of hundreds of meters of polyethylene barriers, and annual actions to support metamorphosis by preventing egg masses and tadpoles from drying from the too rapid retreat of water. The effect of frogs patrolling actions and safeguarding reproduction through egg masses and tadpoles rescuing (all with authorization from Italy Environment Ministry) has led to a progressive strong increase in egg depositions of *Rana dalmatina*, reaching the exceptional number of 21,200 egg masses in 2025 representing a 254,64% increase compared to previous years.

ECOLOGY

Topsy-turvy behavior of a relict Apennine population of *Vipera ursinii ursinii* (Central Italy): a way to cope with climate change pressure?

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Abstract

Vipera ursinii is a Viperidae of high conservation and naturalistic value. In fact, it is one of the snakes species most at risk of extinction in Italy, where is restricted to summit areas of Central Apennines. It's particularly threatened by Climate change, which makes thermoregulation, prey hunting and reproduction increasingly difficult. M.A.I.A. Project (Monitoring of habitats and animal species affected by climate change) has been active since 2024 with the following objectives: (a) to update and expand knowledge on the distribution and conservation status of *V. ursinii* in Maiella National Park; (b) to evaluate the impact of climate change on known populations. This Project is funded by the European Union - Next Generation EU, Mission 4 Component 2 Measure 1.4 CUP B83C22002930006. All activities were authorized by MITE 0068141.31-05-2022 and MASE 0078036.24-04-2025. Individuals were manually captured, tagged with PIT tags and recaptured to estimate the local population using the Lincoln Petersen index. A subset of adult individuals have also been marked with HDF diodes, applied to the right flank of the last third of the body, and subsequently detected using RECCO technology. Genomic data will be integrated into the ERGA project. To better understand local population distribution, drones were used to generate a detailed map through photogrammetry. In 2024, 10 females and 6 males were marked, and 5 of them were recaptured, resulting in an estimated population of 46-47 individuals. Interestingly, no juveniles were captured during monitoring in 2024. Field observations of HDF-marked individuals in 2025 revealed unusually high levels of elusiveness compared to other Apennine populations, with a predominance of thermoregulation and diurnal activity conducted under natural shelters or within surface burrows of voles. This behavior appears not to be linked to predator presence but rather to the surface microclimate conditions.

BIOGEOGRAPHY

Anthropogenic translocations could explain contrasting patterns of genetic variation in the historically traded Ibero-Maghrebian Mediterranean pond turtle, *Mauremys leprosa* (Testudines: Geoemydidae).

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Abstract

The Mediterranean Basin has been subjected to geological and paleoclimatic events that shaped the biogeographic patterns of many thermophilic taxa. Alongside these natural events, humans have also played a significant role in shaping the distribution of many species, especially those that have long been culturally related to humans. We combined multilocus genetic and environmental data to assess the relative role of climate and geographic barriers on the biogeography of the Western Mediterranean Pond turtle, *Mauremys leprosa*, a chelonian widely distributed throughout the Iberian Peninsula and the Maghreb region that has been historically traded by humans. Our results show (1) high levels of genetic diversity and structure within populations located across the Atlas Mountains in Morocco, and (2) shallow patterns of contemporary genetic structure among Iberian populations. Moreover, we identified one individual in the Iberian Peninsula that likely originated from a population situated across the Atlas Mountains. The absence of genetic structure across the Iberian Peninsula may result from the mixing effects of human-mediated translocations. We emphasize the importance of genetic characterization for the conservation of species that have been historically traded by humans, as is the case for chelonians.

ECOLOGY

Amphibians at the interface between lowland and mountains: the use of Joints Species Distribution Models to study environmental drivers of distribution and define Regions of Common Profile

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Abstract

Species Distribution Models (SDMs) are a widespread methodology in ecology and can be a useful tool for several ecological applications, e.g.: niche study, biogeography, conservation.

Joint Species Distribution Models (JSDMs) are a multispecies extension of SDMs which allows to consider interspecific interactions while modelling species-specific responses to environmental factors. JSDMs can also provide the basis for grouping species according to their probability of being found in each unit of the study area, thus mapping Regions of Common Profile (RCPs).

In this work we applied JSDMs, in the framework Hierarchical Modelling of Species Communities (HMSC), to 10 amphibian species at the interface between the lowland area of the Po Valley and the hilly and mountainous one of the Northern Apennines, Italy.

The aims were: assessing species-specific importance of environmental drivers of distribution; testing species interactions analyzing residual cooccurrence patterns; individuating RCPs; updating distribution and role in the species assemblages of the alien *Pelophylax ridibundus*.

Among the considered environmental variables the ones most affecting amphibian distribution were mean annual temperature, cover of broadleaf forests, and vineyards. Species-specific differential responses have been found both in the direction and the entity of the effects. We didn't find support for any biotic interaction but the cooccurrence pattern allowed discriminating species based on the type of breeding site (streams vs ponds). We identified three RCPs: a lowland one with farmland

adapted species; a transitional one with widespread species; a purely Apennine one, mainly grouping specialist species. The first two regions had *P. ridibundus* itself as the dominant species.

The study supports the applicability of the HMSC framework to amphibians and the definition of RCPs on a regional scale. The obtained results can be a useful basis for conservation actions and long-term studies.

MORPHOLOGY

Colors in mom's food: Carotenoid content in the eggs of an obligate egg-feeding frog

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Abstract

Poison frogs produce bright coloration through multiple mechanisms, including the dietary sequestration of carotenoid pigments, which impart yellow, orange, and red coloration. Preliminary data with a captive frog colony at Goethe University demonstrates that freshly metamorphosed *Oophaga pumilio* contain largely identical carotenoids to their parents and have the same intense red coloration. This is remarkable given that their only source of dietary carotenoids is the trophic eggs provided by the mother, as *O. pumilio* are obligate egg-feeders. To investigate further, we analyzed *O. pumilio* eggs using basic carotenoid extraction, spectrometry, thin-layer chromatography, and HPLC-DAD-MS. Initial results confirmed the presence of beta-carotene in the eggs, implying a direct dietary carotenoid source for offspring and suggesting a mechanism for intergenerational transmission of aposematic coloration. Future analyses will compare these findings with non-obligate egg-feeding species (*Ranitomeya sirensis* and *Epipedobates anthonyi*) to assess differences in carotenoid composition.

CONSERVATION

Thirty-eight years of monitoring brown frogs in the Øyeren delta in Norway

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Abstract

Background: In 1988 Nordre Øyeren Biological Station started to map the habitat selection and distribution of amphibian species in the Øyeren delta. The work started up with the intention of using the results in a future Master thesis (cand. scient).

Landscape and metapopulations: The Øyeren delta covers 7,5 km² of land, and is a bird-foot delta on the river Glomma. The catchment area for the delta, is around 40,000 km². Nine metapopulations occur, separated by tributaries and surrounded hills.

Habitats and waterholes: The delta is covered with grassland, bushes and forests, with annual floods that provide the main source of water. Most waterholes (defined as a potential spawning site for amphibians) are natural, and vary in its structure and appearance from the delta process' that created them. A total of ca. 170 waterholes have been identified, in which three metapopulations are monitored annually.

Monitoring reproducing females: The measure for population size, is the number of reproducing females. They are surveyed by counting number of egg clusters. An accurate and efficient method, but requires frequent visits during a short season.

Monitoring habitats: Though time, we have learned that population size of the brown frogs fluctuate tremendously between years, and that it is the food resources of the habitat that usually limit their numbers. Furthermore, due to the high number of eggs laid, populations recover fast after short term catastrophies.

Rather, it is the habitat availability and size, that is the true threat to local populations. The structure and distribution of terrestrial and aquatic habitats within a metapopulation, is the true limitation for population size and their long-term survival. So during more recent years, we have gradually included techniques for monitoring habitats and habitat threats. They include standardized photographic methods, using SLR camera, sportscamera (e.g. GoPro) and areal photographs taken regularly by the

OTHER

New color-variation in the Moor Frog *Rana arvalis* discovered in the Øyeren delta, Norway

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Abstract

Rana arvalis is known to have several color variations within its distribution. From the Scandinavian peninsula *R. arvalis* forma *striata* and forma *arvalis* are described from the district of Umeå and Skåne. However, no data on color variation has been collected from the Oslofjord region.

During the spring of 1993 *Rana arvalis* was caught in two waterholes on Gjørholmen (n=55). The males seemed to be entirely light blue during spawning. The color appeared 1-2 days after the individuals arrived at the spawning site, only to remain for 2-3 days. Therefore, most male individuals caught were partly blue or lacking such color.

Most individuals had an indication of a stripe on their throat. The stripe on the males' throat was formed by the lack of blue color pigments along a narrow median line (100%, n=19). After the mating season the blue pigments vanished from the throat, resulting in the stripe fading or almost disappearing. On most individual males it was very difficult, or impossible, to detect the stripe after spawning.

The females had a similar stripe on their throat (94%, n=18), but this was a result of marbling, rather than color pigments. The females therefore kept their stripe after spawning as well. The color patterns seem to be sex-biased (100%, n=37).

Gislén and Kauri mention that most *Rana arvalis* have a white underside. Only in relatively few cases may there be cloudy spots occuring on the belly. On Gotland the greyish spots may appear on almost the entire underside. In some cases only (from Västergötland, Skåne and Blekinge Torhamn) there is a uniformly dark colored throat with a white medio-ventral line. Nilsson and Andrén found that 100 % of the Gotland frogs had this white medio-ventral line. However, all had marbeling on their belly. Elmberg mentions that this "throat stripe" is very rare and he only found it on one individual south of Umeå. From Øyeren no frogs had spotted underside, except for the throat.

We have chosen to call the color variation in Øyeren for forma *oeyereniensis* since no name has been given before. The findings from Øyeren seem also to differ somewhat from the Gotland population. However, we are interested in observations elsewhere to confirm whether or not some of our findings are unique to the Øyeren/Oslofjord area.

ECOLOGY

Preliminary results on reproductive characteristics of European pond turtles in Serbia

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Abstract

Unlike many other vertebrate taxa, the body of most chelonians is enclosed in a rigid shell. The turtle's shell physically limits changes in volume of the internal organs, including changes related to a developing clutch. To explore the relation of maternal body size and clutch size in the European pond turtle (N = 51) from five wild populations in Serbia, we measured shells of gravid females using caliper, and counted and measured the eggs using X-ray imaging. The shell and egg volumes were calculated using the modified formula for an ellipsoid: $\pi \times \text{length} \times \text{height} \times \text{maximal width} \div 6000$. The total clutch volume in the overall sample varied between 5.5% and 13.8% of the maternal body volume. The females of the different populations differed in terms of the percentage of the body volume occupied by their clutch (ANOVA $F = 3.99$, $p = 0.007$). Clutch size varied between 2-16 eggs, which correlated strongly and significantly with maternal straight carapace length ($r(50) = 0.862$, $p < 0.001$). One of the explored turtle populations, Rudinje, is characterized by a rather small body size. Compared to other populations, these turtles carry fewer, rather elongated eggs. These results fit well with the observations that large-bodied turtles have larger clutches with round eggs, while smaller turtles tend to produce smaller clutches with elongated eggs. Our results suggest that adult body size determines the variability of clutch size and egg shape in European pond turtles.

CONSERVATION

Stripes in the city: Tracing back non-native Grass Snakes (*Natrix natrix*) in Berlin, Germany**Frederic Griesbaum**

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Humboldt Universität zu Berlin

Abstract

Recent and historical observations of striped Grass Snakes (*Natrix natrix*) in urban Berlin challenge the expected morphological and genetic patterns of local populations. These atypically colored individuals, displaying pronounced dorsolateral striping, characteristic for the southeastern European subspecies *N. n. moreotica*, were found in isolated anthropogenic habitats such as the Britzer Garten and Spreepark—locations with long histories of public access and habitat modification. While formerly interpreted as rare natural variants, the spatial clustering of such morphotypes and their recurrence across age classes (including hatchlings) suggest the presence of reproducing, non-native genotypes.

Building on earlier morphological findings, our current research integrates field surveys and molecular genetics to investigate the origin and potential introgression of these allochthonous individuals into Berlin's native populations of *N. n. natrix*. Preliminary findings, supported by historical accounts of pet trade releases and known introduction events elsewhere in Central Europe, indicate a likely anthropogenic origin. The project aims to genetically characterize individuals from five urban subpopulations using mitochondrial markers and microsatellites, and to assess hybridization with local lineages.

This study not only contributes to the understanding of cryptic urban biodiversity and human-mediated dispersal but also raises important conservation concerns regarding genetic pollution and the long-term integrity of native herpetofauna. The outcomes are expected to inform urban wildlife management and contribute to policy development on invasive reptiles in European cities.

CONSERVATION

Occupancy Modelling Reveals Key Drivers of Amphibian Habitat Use in German Mining Sites

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Abstract

Under increasing pressures of urbanisation, large post-industrial sites such as mining sites can therefore add to the current knowledge of regional species occurrences. Modelling species occupancy in mining sites can thus contribute significantly to understanding and quantifying species-specific habitat change. This poster explores diverse factors driving amphibian diversity in German mining sites by looking at their impact on species-specific habitat use. The amphibian diversity in 88 waterbodies at limestone, gypsum, sand and gravel mining sites was assessed through transect walks and eDNA sampling. Subsequently, occupancy models were fitted to species-specific occurrences in response to waterbody size, hydroperiod, successional stage and depth. Species-specific effects of waterbody age, size, depth, and fish presence were found at the waterbody-level, while waterbody abundance and permanence were found to affect amphibian richness at the landscape level. Further, the waterbody-level and landscape-level effects were found complexly interlinked. The mining sites in this study supported a high amphibian diversity, making them of high conservation potential and supporting the need to further study biodiversity in mining sites. The results may be especially important for fragmented populations in rapidly declining natural habitats. Moreover, they could help practitioners in nature conservation and the mining industry devise general management concepts for active and abandoned mining areas.

ECOLOGY

Characterizing the femoral gland secretion proteome in the lek-mating Galápagos marine iguana (*Amblyrhynchus cristatus*) using bottom-up quantitative proteomics

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Abstract

Femoral glands are epidermal glands arranged ventrally on the hind limbs of many squamate taxa. Their secretions are a mixture of a lipophilic and proteinaceous fraction and are assumed to serve as a chemical signal facilitating inter- and intraspecific communication, especially in the context of mate choice and territoriality. However, the composition and biological function of those secretions, particularly of the much larger protein fraction, are poorly characterized and for most taxa completely unexplored. In this study, we aim to investigate the protein composition and function in femoral gland secretions by studying one of the most extraordinary reptile species: the Galápagos marine iguana (*Amblyrhynchus cristatus*). Being the only described lek-mating reptile and endemic to the remote Galápagos archipelago, the marine iguana provides an ideal system for analyzing the function of femoral gland secretions as a mating-associated signal under natural conditions. We sampled femoral gland secretions of 33 territorial male individuals on San Cristóbal Island during the mating and post-mating season and compared the secretions proteomes using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to identify changes in protein expression during the mating season. Our results show that femoral gland secretions differ in their protein expression between mating and post-mating season. The identified proteins show a broad spectrum of biological functions, including tissue development, immune regulation, response to nutrients as well as

cellular transport functions. We suggest that the femoral gland secretions of the marine iguana have a specialized function during the mating season and might be used as an honest signal for mate choice and territory establishment through signaling male attributes. This will be addressed by combining these molecular results with collected behavioral ecological data of marine iguana lek-mating.

CONSERVATION

Nesting Ecology of the Invasive Pond Slider (*Trachemys scripta*): Behaviour, Nest Site Choice and Temperature-dependent Sex Determination**Olivia Hartmann**

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Freshwater ecosystems are one of the most biodiverse habitats but are threatened by various factors. Invasive species are one of these factors and pose a significant threat to global freshwater biodiversity, with the North American terrapin *Trachemys scripta* being one of the most invasive reptiles worldwide. Introduced through the pet trade in the mid-20th century, *T. scripta* has established itself in Europe, where it competes with native species such as *Emys orbicularis*, and disrupts ecosystems. In this study, I will investigate nesting ecology of *T. scripta* by focusing on preferred nesting sites and sexual maturity of reproductive females. In addition, I will examine resulting sex ratios in nests by integrating abiotic factors and research on temperature-dependent sex determination (TSD), a mechanism where incubation temperature decides the offspring sex. Based on these factors, I will develop a model to assess resulting sex ratios at different sites across Europe. Combining empirical data with field observations will provide new insights into reproductive success and population growth of *T. scripta*. This can further aid population dynamic models and direct future management options, such as nest removal, supporting conservation efforts of native biodiversity in Europe.

MORPHOLOGY

Developmental sequence of the chondrocranium in the obligate carnivorous larvae of *Lepidobatrachus laevis* (Amphibia: Ceratophryidae)

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Abstract

The vertebrate head and its skull represent a significant innovation that has played a key role in the evolutionary and ecological success of vertebrates. It is essential to have reliable information on the development of cartilage structures in a wide range of vertebrate species. Therefore, we studied the cranial chondrogenesis of the larva of the Budgett frog, *Lepidobatrachus laevis* (Ceratophryidae, Neobatrachia). We applied several methods, including histological preparation of transverse sections of the chondrocranium, morphological analysis of three different states of development (mesenchymal aggregation, differentiation, and chondrification), and three-dimensional digital reconstructions. As a result, we observed that the Anlage of the chondrocranium at Gosner stage 19 is laterally compressed, that is, it is initially higher than wide. It gradually flattens, enlarges, and differentiates until reaching a very wide and flat shape at Gosner stage 26. Furthermore, we show that the chondrocranial development of *L. laevis* takes place in a mosaic pattern, which differs to non-tetrapod vertebrates in which an anterior to posterior gradient of chondrification is observed. We identified 19 developmental stages in *L. laevis* according to the chondrification state of its cranial structures. The first element reaching the differentiation-into-chondroblast stage is the hypobranchial plate of the branchial basket, and chondrification, that is, final differentiation, occurs simultaneously in several structures of the neurocranium and viscerocranium. We hypothesize that the rapid chondrification of *L. laevis* chondrocranium is an adaptation to the semi-arid climate conditions from its type locality, El Gran Chaco in South America. Due to the only temporary availability of suitable water accumulations this apparent accelerated development would make sense to ensure the tadpoles are froglets by the time the water body disappears.

BIOGEOGRAPHY

Exploring Paleogene Tibet's Warm Temperate Environments in Himalayan spiny frogs

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Abstract

The Cenozoic topographic development of the Himalaya-Tibet orogen (HTO) substantially affected the paleoenvironment and biodiversity patterns of High Asia. However, concepts on the evolution and paleoenvironmental history of the HTO differ massively in timing, elevational increase and sequence of surface uplift of the different elements of the orogen. Using target enrichment of a large set of transcriptome-derived markers, ancestral range estimation and paleoclimatic niche modelling, we assess a recently proposed concept of a warm temperate paleo-Tibet in Asian spiny frogs of the tribe Paini and reconstruct their historical biogeography. That concept was previously developed in invertebrates. Because of their early evolutionary origin, low dispersal capacity, high degree of local endemism, and strict dependence on temperature and humidity, the cladogenesis of spiny frogs may echo the evolution of the HTO paleoenvironment. We show that diversification of main lineages occurred during the early to Mid-Miocene, while the evolution of alpine taxa started during the late Miocene/early Pliocene. Our distribution and niche modelling results indicate range shifts and niche stability that may explain the modern disjunct distributions of spiny frogs. They probably maintained their (sub)tropical or (warm)temperate preferences and moved out of the ancestral paleo-Tibetan area into the Himalaya as the climate shifted, as opposed to adapting in situ. Based on ancestral range estimation, we assume the existence of low-elevation, climatically suitable corridors across paleo-Tibet during the Miocene along the Kunlun, Qiangtang and/or Gangdese Shan. Our results contribute to a deeper understanding of the mechanisms and processes of faunal evolution in the HTO.

ECOLOGY

Earlier maturation or egg retention in tree lizards responding to predation risks in different stages

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Abstract

Predation risk is a significant selective force in the evolution of reproductive characteristics of prey. Prey confront multiple predators, leading to varied predation risks and triggering defensive responses in traits and behavior. However, rare studies have examined whether different reproductive strategies in response to different predation risks, such as predators of adults versus offspring in lizards. Most studies often focused on a single risk type, neglecting mixed risk scenarios. Here, we utilized the reburied lizard clutches and clay lizard model experiments, each conveying different predation risks. We also examined how females respond to their reproductive strategy and age of maturity under the influence of predation risk. We reared the pregnant *Diploderma swinhonis* females captured from eight populations of Taiwan to assess how differences in predation risk associated with the morphological traits and reproductive strategies. The results showed that females' reproductive strategies are associated with predation risk. We found that in Kenting, females with highest avian predators reproduce earlier but lay more eggs with higher hatching rates per clutch. This suggests that females may invest more energy into reproduction by maturing earlier. Females from Orchid Island's population with the highest density of egg-eating snakes exhibit larger body sizes and later reproductive ages. Furthermore, 20% of these females tend to lay an additional clutch in a different location on the same day, and the eggs from this clutch represent relatively long periods of egg retention and relatively short periods of hatching. This implies that female lizards may reduce egg predation risk by not only increasing clutch number but also reduced the hatching period. This study helps to understand how lizards employ flexible strategies to cope with different predation risks. These adaptations balance reproductive costs and benefits according to perceived risks.

BIOGEOGRAPHY

Filling Data Gaps on Georgian Herpetofauna: The Power of Citizen Science on Social Media, Focusing on Snakes

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Abstract

Effective snake conservation in Georgia, a biodiversity hotspot, is hindered by data gaps on species distribution and ranges. Citizen science, via social media, offers a powerful tool to address these knowledge gaps by engaging the public in data collection. Understanding precise ranges of Georgia's 22 recorded snake species is vital.

This study analyzed citizen science data 2017–2019 via social media (FB, iNat, GBIF, etc.), alongside historical fieldwork and publications. Data included location (± 1 km), time, and photographic evidence for identification. Data analyzed using 10x10 km squares (AOO) and convex polygons (EOO) to assess increases and effectiveness. Data collection and analysis are ongoing, involving a significantly larger network of contributors, leading to a continuous increase in recorded locations and promising even better results.

Integrating new data increased known locations by 78%, leading to substantial gains in estimated EOO (up to 269%) and Area of Occupancy (up to 188%). Notable range expansions ($>100\%$ EOO increase for *Elaphe dione*, *Zamenis hohenackeri*, *Hemorrhois ravergieri*, lowland *Vipera eriwanensis*) were observed. Over 100% AOO increase for species like *Zamenis longissimus*, *Vipera kaznakovi*, and the lowland form of *Vipera eriwanensis*. Furthermore, social media data correlated strongly with AOO gains ($r=0.92$) and associated with mobility. While traditional fieldwork was more effective for less mobile species, social media proved valuable for documenting mobile Colubrids and revealing new locations away from traditionally surveyed sites, contributing to more even geographic distribution.

Citizen science via social media is a valuable, complementary approach to traditional methods for snake monitoring in Georgia. Critical for refining conservation status assessments (e.g., IUCN) and guiding future research and conservation efforts. This approach is particularly valuable in data-deficient regions like Georgia.

CONSERVATION

From one species to a national network: the development of amphibian ex situ conservation in Brazil**Renata Ibelli Vaz**

Amphibian Ark

Corresponding author: ibelli.renata@gmail.com**Abstract**

Brazil holds the highest amphibian diversity on Earth, yet structured ex situ conservation programs remain limited. The first initiative began in 2009 at the São Paulo Zoo with *Ololygon alcatraz*, a microendemic species that was then listed as Critically Endangered. Thanks to combined in situ and ex situ efforts, the species was later downlisted to Vulnerable on the IUCN Red List. In 2020, a Conservation Needs Assessment (CNA) for Brazilian amphibians identified 14 species as rescue priorities, requiring urgent ex situ efforts due to imminent extinction risks. However, progress on amphibian ex situ programs was slow, with a second initiative beginning ten years later, with the species *Nyctimantis pomba*. As of 2025, five threatened species are under ex situ conservation programs in Brazil: *Physalaemus signifier*, *Melanophryniscus setiba*, *Atelopus manauensis*, *Pithecopus rusticus*, and *N. pomba*. These programs are led by different zoological institutions in collaboration with academic researchers and supported by the Amphibian Specialist Group (ASG), Amphibian Ark, and local governmental institutions. Despite progress, significant challenges remain. A national survey revealed major barriers, including inadequate infrastructure (5%), shortage of trained personnel (3.3%), and institutional or bureaucratic constraints. One of the most concerning challenges is the scarcity of professionals with practical experience in amphibian husbandry, veterinary care, and long-term conservation planning. To address these barriers and to expand and strengthen ex situ efforts, Amphibian Ark launched a capacity-building initiative in 2023. This includes technical training, species prioritization, institutional engagement, and support for conservation planning. All these initiatives show significant developments in establishing conservation efforts in Brazil; however, there is still a need to expand the scope of preserved species through ex situ programs.

SYSTEMATICS

Comparative analysis of mitochondrions in European water frogs of the genus *Pelophylax*

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Abstract

The moderate evolutionary rate of vertebrate mitogenomes renders their genes suitable for constructing models in phylogenetic studies. The comprehensive coverage of the entire mitochondrion allows for a more detailed reconstruction of phylogenetic relationships within taxonomic groups. The molecular phylogeny of water frogs of the genus *Pelophylax* closely related to the widespread invasion of the marsh frog, which represents a series of cryptic species with unresolved taxonomic status, as well as the molecular mechanisms induced genome elimination in hemiclinal species. Currently, there are nearly 50 complete mitochondrial genomes in genetic databases for Western Palaearctic water frogs. In the recent study, we are adding data on 30 mitochondrial genomes of water frogs from the Caucasus, the Volga drainage region, Saint Petersburg, and Moscow, obtained through next-generation sequencing.

The total length of mitochondrions in water frogs averages 17,900 bp. An analysis of 47 mitochondrions from western Palaearctic green frogs of the genus *Pelophylax* revealed 4,190 mutations, 3,684 polymorphic sites, and 1,474 singleton mutations. 36 haplotypes were identified. The highest levels of nucleotide and haplotype diversity were observed in genes encoding NADH dehydrogenase, cytochrome c oxidase, and cytochrome b. The ribosomal RNA genes exhibited the high variability in the pool frog, *P. lessonae*. The overall topology of Maximum Likelihood and Bayesian phylogenetic trees is consistent, with some exceptions at specific positions within clades. The largest uncorrected genetic distance was noted between *P. cretensis* and *P. shqipericus* (0.129), while the smallest was observed between *P. ridibundus* from the Volga region and Europe (0.001). The Central European clade of the marsh frog is characterized by the lowest levels of genetic variability, whereas the highest levels were recorded for Anatolian *P. cf. bedriagae* that includes several undescribed cryptic species.

OTHER

The new Handbook of Zoology (Reptile volumes)

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Abstract

The Handbook of Zoology (De Gruyter, publishers) has a tradition of over 100 years now. The last reptile volumes appeared in the 1950s, so a new edition seemed high time. A first volume appeared in 2024, a second volume is planned and will appear soon.

Volume 1 contains 10 chapters written by 12 renowned authors: on embryology and life history, the skeleton of reptiles, the cardiovascular system of reptiles, the Paleozoic origin of major reptile groups, Chelonia, and four chapters on archosaurs, including crocodylians, dinosaurs and pterosaurs.

In the second volume, the lepidosaurs as well as general aspects of biology such as skin structure, thermobiology and snake venom will be treated.

CONSERVATION

Life history traits of amphibians and reptiles in agricultural habitats

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Abstract

Life-history data on amphibian and reptile populations inhabiting agricultural habitats are limited. To help fill this knowledge gap, a life history traits database for European species was compiled. Species were selected based on their presence in the following habitat types: agroforestry, dry, irrigated, pastures, and woody habitats. In total, 18 amphibians (13 anurans and five urodeles) and 43 reptiles (30 saurians, nine snakes and four turtles) were included. Data for 29 traits were extracted from the literature. For each trait, susceptibility level to pesticide exposure was evaluated and categorized as individual, population, or both. Species were ranked according to the amount of data available. Among amphibians, the agile frog (*Rana dalmatina*) had the most data available, followed by the common frog (*R. temporaria*) and the common toad (*Bufo bufo*). Among reptiles, the species with the most data available was the smooth snake (*Coronella austriaca*), followed by the European pond turtle (*Emys orbicularis*) and the green whip snake (*Hierophis viridiflavus*). This database highlights a significant lack of basic information necessary to protect these species from the harmful effects of agrochemicals. However, it also provides valuable qualitative and quantitative data that can serve as a foundation for identifying focal species in future risk assessments.

OTHER

Pet trade of amphibians in Poland: comparison after 10 years

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Abstract

One of the causes of amphibian decline is the over-exploitation of wild populations for various purposes. Many species are increasingly being captured and used as pets. The aim of our research was to investigate the current market for exotic amphibian trade in Poland, to identify changes that have occurred over the last decade. From October 1, 2023, to September 30, 2024, data on amphibians availability in the Polish market were collected by analyzing online resources and offers from terraristik fairs. A total of 630 offers were recorded, covering 113 species, including two native species (*Triturus cristatus* and *Ichthyosaura alpestris*) and one hybrid *Ceratophrys cranwelli* x *cornuta*. The offered amphibians represented two orders: Anura (91 species) and Caudata (22 species). The predominant families were Bufonidae, Dendrobatidae, Hylidae and Salamandridae. These families garnered the greatest interest, primarily due to their vibrant coloration or relatively large body size. Of the animals offered, 37.3% were listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). At least 20% of the offered species were captured from the wild based on sellers' declarations, and one species *Xenopus laevis* is classified as invasive alien species and poses a threat to European fauna. Compared to our previous data (2013-2014), there was a slight increase in the number of species offered (by one), while the number of offers increased by as much as 73.5%. The similarity between the two study periods was low. Only 35.8% of species were common to both. The proportion of endangered species also increased (from 12.5% of all species offered to 19.5%). The results indicate a continuing increase in interest in keeping amphibians as pets. Many species are still being captured from the wild, which poses a threat through over-harvesting of wild populations and the risk of introducing foreign pathogens.

ETHOLOGY

Colour signalling and sexual dichromatism in two sister species of ocellated lizards.

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Abstract

Animal colours serve various ecological and social functions, including signalling, thermoregulation, camouflage and predator deterrence. The genus *Timon* (Lacertidae) represents the largest lizards in Europe. Besides their size, they are characterised by their complex colour patterning, including ultraviolet (UV)-blue lateral ocelli. Previous research highlighted cryptic sexual dimorphism in *T. nevadensis* in the UV, linking male coloration to body size and suggesting a potential signalling function. Such traits may play a role in intraspecific communication, with larger males signalling higher fitness or dominance through their patches. Although historically considered as subspecies, *T. lepidus* and *T. nevadensis* are now recognised as distinct species based on genetic data. They also show clear differences in colour patterning and habitat preferences: *T. nevadensis* has a silver-grey appearance and inhabits semi-arid environments, while *T. lepidus* is vivid green and found in denser, vegetated habitats. These divergent habitats may influence not only the overall coloration but also the visibility and ecological function of UV-blue signals. In this study, we used multivariate analyses and visual models to analyse the spectral properties, sexual dimorphism, and conspicuousness of UV-blue patches and explore intra- and interspecific variations. Our findings reveal that while both species share some visual traits, *T. lepidus* exhibits stronger sexual dichromatism and fewer but more conspicuous patches, whereas *T. nevadensis* shows a higher number of larger patches, yet less conspicuous ones. These patterns may represent alternative signalling strategies shaped by species-specific habitats.

Our results highlight the role of environmental context in shaping visual signals, providing insights into the evolution of UV-based traits in these two lacertid species.

OTHER

Dissecting without damage: Inferring taxonomic affiliations from the gut content of a preserved museum specimen

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Abstract

Natural history collections are highly valuable for taxonomic research, as they offer important records of biodiversity. Studying the internal structures of these specimens has previously been limited by the need for dissection, an irreversibly destructive method that is not applicable for valuable specimens. The introduction of micro-computed tomography (μ -CT) made it possible to visualize osteological features in 3D without damaging the specimen. Various staining techniques further allowed the visualization of soft tissues but often caused minor damage by altering specimen color or compromising the DNA. In contrast, recent two-phase μ -CT scanning enables a non-destructive approach and preserves the original condition.

We used two-phased μ -CT data to examine the stomach content of *Plethodontohyla angulifera* Werner, 1903 (Anura: Microhylidae: Cophylinae), a more than 120-year-old holotype of a species, which is currently considered a nomen inquirendum. The specimen is in poor condition, preventing a reliable morphological assignment within cophylina frogs. We analyzed the stomach contents to narrow down its taxonomic identity as tissue sampling for molecular identification was not permitted. Since the original label only stated 'Madagascar' as the locality, we aimed to infer a more precise geographic origin based on the biogeography of its prey. Segmentation of sclerotized remains in the stomach revealed a strongly myrmecophagous diet, with over 14 ant heads and 9 thoraxes from two subfamilies within Formicidae. Advanced visualization tools allowed us to virtually reassemble prey specimens, facilitating identification at the genus level based on key phenotypic traits and allowing an approximate geographic inference. Our study underscores the remarkable variety of information that can be obtained from historical museum specimens, even in the absence of morphological and genetic data, and emphasizes their relevance for taxonomic and biogeographic research.

ETHOLOGY

Just a light touch: The way of transfer of potential pheromones during cephalic amplexus in poison frogs

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Abstract

Chemical communication is an ancient and widespread mode of information transfer which has been documented in numerous animal groups, including amphibians. Chemical signals serve various functions in interspecific and intraspecific interactions, such as mediating social behaviors, marking territories, or facilitating mating and courtship. While in urodelians (newts and salamanders), chemical communication during courtship has been well studied, in anurans (frogs and toads) many aspects remain poorly understood. It is, however, known that the males of several anuran species produce Sodefrin Precursor-like Faktors (SPFs) in specialized breeding glands – proteins which are known to act as courtship pheromones in urodelians. In the Neotropical poison frog species *Epipedobates anthonyi* (Dendrobatidae), it was recently discovered that males produce SPFs in specialized breeding glands in their fingers IV. These fingers are brought into close contact with the female's head during cephalic amplexus – a type of amplexus only found in poison frogs. It is, however, still unclear, how exactly SPF is transferred from the male's fingers to the female, and if the proteins are delivered over the nostrils (which would classify them as pheromones) or if they are transmitted transcutaneous, omitting the sensory organs (which would classify them as socially transferred materials). To solve this question, we investigated the mechanisms of potential SPF transfer by recording mating events of captive *E. anthonyi* and analyzing the positioning of the males' fingers during amplexus, using the software BORIS. We further investigate, how long the frogs remain in amplexus, and if the duration of positioning the fingers at the female's head is correlated to breeding success.

CONSERVATION

Limbless squamates dominate among road-killed reptiles in Poland

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Abstract

The development of road infrastructure significantly contributes to the fragmentation of animal habitats and, consequently, to increased wildlife mortality due to vehicle collisions. This is considered one of the major factors contributing to the decline in populations of small vertebrates. Although road mortality commonly affects reptiles, the scale of this phenomenon remains relatively poorly understood, especially when compared to more extensively studied groups such as amphibians. The aim of this study was to summarize literature data on reptile road mortality in Poland. We found data from 16 systematic studies published in scientific literature and conference proceedings between 1978 and 2023, of which four provided incomplete results or focused solely on a single species, and were therefore included only in selected analyses. The proportion of reptiles among all road-killed vertebrates ranged from 2% to 66%, depending on the study. A total of 2,066 dead reptiles representing six species were recorded. The most frequently affected species was the grass snake, *Natrix natrix* (70.8%), followed by the slow worm, *Anguis fragilis* (9.7%), the European common adder, *Vipera berus* (7.8%), and the sand lizard, *Lacerta agilis* (6%). Limbless squamates (88.8%) significantly dominated over limbed reptiles (11.2%). The highest mortality rates were observed in spring and autumn, likely reflecting seasonal migrations of reptiles. Most reptiles were found dead near wetlands and forest habitats, while relatively fewer casualties were recorded in agricultural areas. In two study areas, reptile road mortality has been continuously documented for at least 20 years. In one of them, the Stawy Milickie nature reserve, a tenfold increase in reptile road mortality has been observed over time. The reviewed studies suggest that reptiles may represent a substantial proportion of road-killed vertebrates in certain areas. These findings highlight the urgent need to develop and implement effective mitigation strategies to reduce reptile-vehicle collisions.

CONSERVATION

Towards improved welfare monitoring of endangered species: health status and stress assessment in captive olms (*Proteus anguinus*)**Janja Laka**

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Abstract

Ensuring the welfare of captive olms (*Proteus anguinus*) is essential for the success of long-term ex-situ conservation for this highly sensitive and endangered amphibian inhabiting subterranean waters of the Dinaric Karst in the Western Balkans. Although previous studies have established leukocyte profiles and neutrophil-to-lymphocyte (N/L) ratios in both recently captured and long-term captive individuals, no significant differences in these parameters have been observed, so methodologies enabling a reliable insight into health status and assessment of a direct stress remain lacking. Our ongoing research focuses on developing non-destructive in vitro assays to evaluate immune function and stress in olms. In addition to standard blood counts, we are incorporating biochemical profiles and hemoglobin measurements using automated analyzers. Plasma bactericidal capacity is assessed via a bacterial killing assay with *E. coli*. To improve accuracy and reduce reliance on microscopy, we are developing a flow cytometry protocols, which will enable automated differential blood cell counts. The protocols are currently under validation using the non-specific fluorescent lipophilic dye DiOC5(3), due to the absence of species-specific antibodies. Furthermore, ELISA-based immunoassays are being introduced to quantify stress biomarkers and explore their relationship with immune function and overall health status during long-term captivity. Since olms are not experimental animals, all assay optimizations are initially performed on the Iberian ribbed newt (*Pleurodeles waltl*), a model urodele species bred in our laboratory. These methods aim to establish a reliable framework for long-term health monitoring and welfare assessment of captive and wild populations of olms.

CONSERVATION

Five year monitoring in the Mulde floodplains, Germany, highlights a hotspot of amphibian diversity and shows its exceptional importance for the conservation of the fire bellied-toad (*Bombina bombina*)

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Abstract

The fire-bellied toad, *Bombina bombina*, was formerly widespread throughout Central and Eastern Europe. In Germany the species' habitat boundary runs through western Saxony, where the remaining populations are critically endangered due to the loss of spawning waters.

The floodplain of the river Mulde around the village of Kollau in the district of Leipzig (Germany), has been known as a hotspot of amphibian diversity since the 1980s. It was shown that despite a long-term deterioration in habitat condition in the three studied water bodies and a collapse in population numbers, an exceptionally high number of eleven amphibian species still occur in the study area. Evidence of reproduction was found for ten species, including several species of high conservation concern. A monitoring project was carried out from 2020 to 2024, particularly focussing on the fire-bellied toad. This study marks the conclusion of the five-year investigation.

Based on the the collected capture-recapture data a population analysis for *B. bombina* was carried out using the program MARK. For the first time, the population in the area was systematically quantified. A correlation was found between the yearly recorded population numbers of the fire-bellied toad in the area and several environmental predictors (average water level of the Mulde river and amount of yearly precipitation).

BIOGEOGRAPHY

Batrachofauna and Herpetofauna of central Montenegro

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Abstract

The study area of Ćeklići, Bjelice, and Cuce lies on the Katunska karst plateau (Montenegrin rugged karst), a limestone–dolomite plateau situated in central Montenegro at 800–1000 m elevation. The climate is moderately continental with Mediterranean influence, featuring heavy rainfall in autumn and winter and dry summers. Vegetation is sparse due to thin soils and intense karstification, consisting largely of degraded shrubland and isolated forest patches.

A total of 21 species were documented in the area. Among these, 6 amphibian species represented 2 orders and 4 families, while 15 reptile species spanned 2 orders and 6 families. Among these, 7 species are Balkan endemics, highlighting the regional uniqueness of the herpetofauna.

According to national legislation, 18 species are legally protected in Montenegro, specifically, 4 amphibian and 14 reptile recorded species. Under the Bern Convention, all recorded amphibians and reptiles are listed in Appendices II or III. Within the EU Habitats Directive, 4 amphibian species and 11 reptile species occurring in the study area are included in Annexes II, IV, or V. According to the IUCN Red List and the national Red List of Amphibians and Reptiles of Montenegro, most recorded species are categorized as Least Concern (LC). However, two species stand out as exceptions: *Triturus macedonicus*, classified as Vulnerable (VU), and *Testudo hermanni*, considered Near Threatened (NT).

EVOLUTION

Combining genomics and paleoclimatic modelling to assess the evolutionary history of Peringueyi's adder (*Bitis peringueyi*)

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Abstract

The evolutionary knowledge on the sidewinder or Peringueyi's adder (*Bitis peringueyi*), a small, dune-dwelling, endemic species to the coastal deserts of Namibia, has been limited to phylogenetic inferences focused for the entire genus *Bitis*, and based only on few loci. In this work, we investigate the evolutionary history of *B. peringueyi* by combining genome-wide population genomic and paleoclimatic modelling inferences. We have sequenced more than 30 samples spanning across its distributional range and provided an evolutionary context by including some samples from sister dwarf adders (i.e. *Bitis caudalis* and *Bitis schneideri*). Thus, we built a SNP dataset containing more than 10,000 unique SNPs, revealing a pronounced genetic structure across *B. peringueyi* populations, indicating limited gene flow and potential historical isolation. Phylogenetic reconstructions consistently identified two major genetic clusters within the species: one comprising the majority of populations, located in the Namib-Naukluft

and Dorob regions, and a second distinct group restricted to the Skeleton Coast region. Paleoclimatic modelling indicated that the species is restricted the Namibian coast. A pattern of expansion in cold periods (LGM) and retraction/isolation in warmer periods (LIG/current) is recognized, with the southern region acting as climatic refugia. The major intraspecific genetic discontinuity therefore, seems driven by climatic events, although habitat limitations (i.e. absence of dune habitats) are likely involved too. Furthermore, limited gene flow between populations across apparently continuous dune desert ecosystems requires further landscape studies. Therefore, this study provides essential baseline data for future evolutionary, taxonomic and conservation research on this species and underscores the importance of combining genomics and climate modelling into the study of desert biodiversity.

OTHER

Tetrodotoxin in harlequin toads (*Atelopus* spp.) – inferring its potential origin from gut and skin microbiome using DNA metabarcoding

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Abstract

Tetrodotoxin (TTX) is a potent neurotoxin known from a wide array of marine and terrestrial organisms of different habitats and trophic levels. TTX mainly serves as a defence against predation. While some bacteria are known to produce TTX, the origin in vertebrates, especially amphibians, remains a matter of debate. Either, these animals produce TTX by themselves or they uptake it via their prey, or they adopt it from (symbiotic) bacteria. Neotropical harlequin frogs, genus *Atelopus*, possess different amounts of TTX in their skin and inner organs. We analysed (1) arthropod prey taxa in freshly collected feces and (2) bacterial composition of both gut and skin samples of 6 species of *Atelopus* from Ecuador to detect potential sources of TTX. Compositions of gut contents revealed large intra- and interspecific differences with diet being predominantly constituted of Hymenoptera. Other common orders include Coleoptera, Lepidoptera and Araneae. However, a bacterial origin of TTX appears more likely given that no TTX-bearing arthropods were detected. Instead, we detected several bacterial OTUs closely related to strains known to produce TTX. Additional work including bacterial culturing will be required to further disentangle the source of TTX in *Atelopus*.

ECOLOGY

Automated acoustic detection of frogs and newts under different conditions: possibilities and limitations

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Abstract

Passive acoustic monitoring (PAM) is a modern approach that is very useful for monitoring species diversity and population size in anuran amphibians, which are among the most vocally active vertebrates. However, PAM also creates a large volume of data, making manual review of records very time-consuming, and the existing models for automated species detection are often unreliable. We used the cloud-based bioacoustics platform Arbimon to compare PAM results from 240 hours of Audiomoth recordings from four habitats (three natural and one artificial) and from a controlled laboratory experiment. We tested the accuracy of Arbimon's Random forest models for three anuran (*Bufotes viridis*, *Hyla orientalis*, *Pelophylax ridibundus*) and one urodelan species (*Triturus ivanbureschi*). For the experiment, recordings were made in four glass tanks with different number of newts (0, 2, 6, 12), as well as a tank with 10 fish (genera *Ancistrus*, *Botia* and *Trichogaster*). The estimated accuracy and precision for each model was as follows: 79%/78% (*B. viridis*), 82%/85% (*H. orientalis*), 73%/75% (*P. ridibundus*), and 69%/55% (*T. ivanbureschi*). The models largely confirmed their usefulness for anuran species, with false positives and false negatives within the respective estimated error margins. However, in two of the natural habitats the outputs for *P. ridibundus* were heavily influenced by non-target sounds, resulting in extremely high percentage of false positives (76.7% and 96.4%). The laboratory recordings confirmed that the model could successfully recognise newt clicks, revealing a presence of 0%, 36.9%, 57.8% and 62.8% for the tanks with 0, 2, 6 and 12 newts, respectively. The model also worked with field recordings, where newt presence was confirmed with funnel traps in all habitats, but results from the fish tank revealed a false positive of 8.7%. We suggest that automated detection can be further improved through machine learning algorithms.

BIOGEOGRAPHY

Distribution and spread of geckos (Gekkota) in Slovenia: Insights from field surveys and citizen science

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Abstract

Geckos (Gekkota) are a globally widespread group of reptiles, with several species introduced outside their native ranges. In Slovenia, three species have been confirmed: the Moorish gecko (*Tarentola mauritanica*), the Turkish gecko (*Hemidactylus turcicus*), and Kotschy's gecko (*Mediodactylus kotschy*). All are presumed non-native in Slovenia, with human-facilitated introductions and natural dispersal from neighbouring populations both likely contributing to their presence—especially in the case of *T. mauritanica*. Until recently, only two localities had been reported in literature—one in Portorož for *T. mauritanica* and one in the Port of Koper for *H. turcicus*. The aim of this study was to comprehensively assess the distribution of geckos in Slovenia. We combined three methodological approaches: (1) direct field surveys in southwestern Slovenia, (2) a public outreach campaign to collect citizen reports via a media article and social media, and (3) a review of online citizen science platforms and databases (iNaturalist and Bioportal). Between July 2023 and February 2024, we documented geckos at 83 new localities, primarily based on citizen reports. Most records originated from the Slovenian Littoral region (Slovenska Istra), especially around Koper, Škofije, and Portorož. *T. mauritanica* was the most frequently observed species, including both nocturnal and occasional winter diurnal activity. *H. turcicus* was reported especially in the Littoral area, but far less frequently. For both species, all life stages were observed. *M. kotschy* was confirmed only once, in the northeastern part of the country, via the Bioportal platform. Our findings indicate that the *T. mauritanica* and *H. turcicus* are more widespread in Slovenia than previously known, highlighting the importance of citizen science in detecting and monitoring non-native, but charismatic species of herpetofauna.

ECOLOGY

Is the ecological gradient a cause or a consequence? An illustration depicts the various gradients in snake fauna and composition in Taiwan.

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Abstract

Competition exclusion, characteristic displacement, niche partitioning, and spatial heterogeneity are mechanisms that help explain how multiple species coexist along ecological gradients. Taiwan, as a diverse offshore island, features a variety of landscapes and climates, including tropical lowlands, various forest types, steep, high mountains, and cirques. The highest peaks can reach nearly 4,000 meters in elevation, contributing to significant spatial heterogeneity. The reptiles and amphibians of the island originate from five potential sources: Okinawa, the Philippines, Zhejiang or Fujian, Guangdong or Vietnam, with some endemic species having uncertain origins. The island is also home to 50 different species of terrestrial snakes. A clear gradient of beta diversity has been observed among these snakes across varying elevations. Our research has examined multiple ecological gradients and ranked the performance of several snake species, assessing their sensitivity to factors such as habitat disturbance, urbanization, evaporative water loss, vision performance, and thermal selection. In this poster, we will compile data on these ecological gradients and rank them according to the different sympatric snake species compositions found in Taiwan. We will also discuss the ecological characteristics and origins of these snakes.

ECOLOGY

Microplastics and their effect on the development and health of filter-feeding larvae

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Abstract

Microplastics (MPs) are persistent environmental pollutants that are widely distributed in freshwater ecosystems. Amphibians, being highly sensitive to environmental contaminants, have been shown to ingest MPs, yet their effects on amphibian development and health remain largely unknown. In this study, we examined the impact of polyethylene MPs on the growth and health of the filter-feeding African clawed frog (*Xenopus laevis*) larvae. Larvae were exposed to an environmentally relevant concentration of polyethylene MPs, both in the presence and absence of CORT to simulate a stress condition. Physiological health was assessed using telomere length and oxidative stress levels as markers of health and ageing, and we additionally measured levels of the glucocorticoid hormone corticosterone (CORT) as a stress indicator. Our findings show that MP ingestion significantly reduced larval body mass, but it does not seem to result in long-term effects regarding the lack of variation on CORT levels, oxidative stress, or telomere length. These results suggest that, although environmentally relevant concentrations of MPs can impair amphibian larval growth, they may not directly affect physiological stress markers or ageing-related parameters.

ECOLOGY

Scale-dependent landscape variables and linear infrastructures influence smooth newt (*Lissotriton vulgaris*) abundance in wetlands of a heavily urbanized lake

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Abstract

Freshwater ecosystem degradation due to land use change is a major driver of global biodiversity loss and amphibian declines, with impacts varying across spatial scales. This study assessed how natural and human-modified land cover affects smooth newt (*Lissotriton vulgaris*) abundance in wetlands around Lake Balaton, a highly urbanized region.

We conducted aquatic trap surveys at 32 wetland sites during the breeding season and quantified land cover within 250, 500, and 1000 m buffer zones. We hypothesized that urban areas, croplands and proximity to roads and railways would negatively affect newt abundance, while wetlands (marshes, swamps, periodically flooded grasslands) and natural terrestrial habitats (grasslands, forests, woodlands) would have positive effects, varying by scale.

Using N-mixture models, we estimated newt abundance and examined its relationship with land use variables. Results showed that wetland cover within 500 m positively influenced newt abundance, likely by supporting metapopulation connectivity and providing suitable breeding habitat. Conversely, cropland cover within 250 m and proximity to roads and railways negatively affected abundance, indicating habitat disturbance and fragmentation effects.

These findings emphasize the scale-dependent and complex nature of land use impacts on amphibians in urban landscapes. Conservation should focus on

maintaining and restoring wetland habitats to support newt populations and reducing disturbances from agriculture and infrastructure. Ensuring connectivity among wetlands and mitigating negative effects of cropland and transportation networks are vital for the long-term survival of smooth newts in this region.

CONSERVATION

Evaluation of predator control efforts implemented to enhance the conservation status of the Hungarian Meadow Viper (*Vipera ursinii rakosiensis*)

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Abstract

The Hungarian Meadow Viper (*Vipera ursinii rakosiensis*) is a critically endangered subspecies endemic to steppe habitats in Hungary and Romania. Increasing predator pressure—particularly from badgers and foxes—has been identified as a key factor limiting viper recovery, despite decades of reintroduction and habitat restoration efforts. As part of the LIFE18 NAT/HU/000799 conservation programme, we implemented targeted predator control in the viper habitats in the Kiskunság National Park and assessed its effectiveness using dynamic occupancy modelling.

Seventy survey plots (50×50 m and 100×100 m) were monitored over five consecutive springs (10 visits per year), yielding 134 viper detections. Predator abundance was assessed via winter and early spring den and nest surveys, as well as annual predator removal records ($n = 2874$ individuals). Multi-season occupancy models were applied to evaluate the influence of predator densities and control intensity on viper colonisation and extinction probabilities.

Model selection revealed that colonisation probability decreased with badger den density but increased with fox control intensity. Extinction probability was positively associated with total predator density and negatively with overall predator removal. These findings support the hypothesis that mesopredator control can facilitate population recovery in this highly threatened reptile.

Our results provide robust evidence that targeted predator management, implemented under LIFE funding, significantly contributes to viper conservation. Predator control should be continued alongside habitat restoration to ensure the long-term persistence of *V. u. rakosiensis* in Hungary.

BIOGEOGRAPHY

Museomics resolves the identity of *Phrynobatrachus brevipalmatus* (Anura, Phrynobatrachidae) from Angola and reveals its unexpected connection with an oceanic island in the Gulf of Guinea

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Abstract

With nearly 100 species, *Phrynobatrachus* is the second most speciose frog genus in Africa. Moreover, several currently recognized species have been shown to form rather species complexes. In addition, multiple new lineages have been detected in the recent years that may represent distinct species. Confirmation of their taxonomic status, however, is hampered by the existence of several potentially conspecific nominal taxa described a century ago that are known only from type material. The current condition of these types and the frequent lack of diagnostic features often prevent their proper morphological assessment. We focused on one of these little-known species, *P. brevipalmatus* (Ahl, 1925) described from Luanda, coastal Angola exactly century ago, which has not been recorded since its original description. We employed an archival DNA approach (museomics) to obtain molecular data from the type specimen. Our results of data from the holotype, previously published sequences, as well as other new data revealed an unexpected affinity of *P. brevipalmatus* to the São Tomé Island endemic *P. leveleve* from which *P. brevipalmatus* differs only by 1.2% in the mitochondrial ribosomal 12S and 16S fragment. The holotype further differs by only 1.8% from an unidentified *Phrynobatrachus* from coastal Gabon. The Gulf of Guinea oceanic islands are well known for harbouring unique, supposedly endemic species. They are thought to have diverged from their mainland relatives in the relatively old past, after crossing the open water on a 'raft' driven by ocean currents. However, the divergence between the mainland and island populations in our *Phrynobatrachus* case is of a more recent age and the results support their conspecificity. This raises the question of whether other seemingly endemic taxa of the Gulf of Guinea islands may also have hitherto overlooked populations on the African mainland.

CONSERVATION

The European Pond Turtle (*Emys orbicularis*): Modeling Present and Future Distributions and Biological Invasion Risks for Best Conservation Strategy in Europe

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Abstract

The European pond turtle (*Emys orbicularis*) is one of the most vulnerable reptiles in Europe, despite its high plasticity and ability to inhabit a wide range of aquatic ecosystems. For better forecasting future conservation strategy, we developed modern GIS-based distribution models, using climate and anthropogenic data (Climond, GAEZ, Human Footprint). We show that the current suitable habitats of the species (~37% of the European surface area) will increase by 1.2 to 3.3 times by 2050, especially in Eastern Europe and at the northeastern edges of the range (Ukraine, the Baltic States, southern Sweden). The main factors of its distribution were mean annual temperature, solar radiation, and moisture parameters. We also showed a correlation

between habitat suitability models and the Human Footprint index ($r = 0.6$), consistent with the occurrence of the species in artificial and urbanized water bodies.

As far as biological invasions are concerned, there was a significant correlation ($r = 0.7$) between distribution models of native *E. orbicularis* and the invasive non native red-eared slider (*Trachemys scripta*), indicating a high overlap in present time that is expected to increase by 1.2 times by 2050. For example, in southern Ukraine (e.g., urban waterbodies of Odesa), *T. scripta* already dominates over the native *E. orbicularis* (accounting for 94% of all recorded turtles). Additionally, in northern regions, the raccoon dog (*Nyctereutes procyonoides*) may also exert pressure on *E. orbicularis* populations, especially through predation on nests. This study highlights the necessity of integrating climatic, anthropogenic, and biotic factors when developing conservation strategies for the European pond turtle. It is recommended to establish new protected areas in Eastern Europe, maintain regular monitoring of invasive species, and integrate distribution modeling into conservation planning practices. Read more here: <https://doi.org/10.1038/s41598-024-71911-4>

CONSERVATION

Assessing the Impact of Edge Effects on *Bombina variegata* Distribution in Western and Eastern Europe Using GIS Modelling

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Abstract

The conservation of endangered amphibians such as the yellow-bellied toad *Bombina variegata* at the edge of distribution is particularly urgent under ongoing climate and habitat change. We conducted a comparative study of factors driving the present distribution of two major genetic lineages of *B. variegata*, namely the western (genetically older; France) and eastern (genetically youngest; Carpathians, Ukraine). Using GIS-based modelling approaches (MaxEnt, NTBox), incorporating climatic and ecological data (GAEZ, CHELSA, STRM, CULTIR, GRS_2000), we show that the distribution of the western populations is mainly driven by annual temperature range (41.9–44.9%), evapotranspiration (up to 36.4%, mostly in humid forest habitats), precipitation of the driest month (31.7%), human land use (27.9%), and habitat fragmentation (14.8%). Western populations persist in agro-landscape mosaic where large humid forest ecosystems have declined, while climatic instability increases extinction risks. The Carpathian populations demonstrate higher demographic stability, their distribution being mainly driven by spring precipitation (22.1%), annual temperature range (21.8%), and to a lesser extend dry season rainfall, evapotranspiration, and plant species richness. A significant limiting factor was human presence (36.4%, with notable presence in populated areas). Eastern populations inhabit forested wetlands with a stable climate, emphasizing the importance of old-grown forests. Importantly, we found that Carpathian and western populations were more dependent on topography (slope - 13.1%), and on agroecosystems and secondary vegetation, respectively. These results highlight the

need for differentiated conservation strategies, with the protection of humid forest habitats and regulation of human pressure in the East, and habitat restoration—especially forested areas— in the West with sustainable management of agricultural landscapes through the establishment of forest buffer zones.

ECOLOGY

Preliminary Insights into the Spatial Ecology of *Bothrops asper* and *Bothrops osbornei* in Northwestern Ecuador

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Abstract

Ecuador is one of the most biodiverse countries in the world. Among this vast number of species, numerous of the world's most misunderstood mesopredators are found: Vipers. Terrestrial and arboreal species often differ significantly across taxa. In Crotalinae, arboreal species are often smaller bodied, slenderer and have adaptations like prehensile tails, terrestrial species tend to be larger. Un Poco del Choco Nature Reserve and Biological Station (UPDC) is embedded in the Pichincha Region of Northwestern Ecuador. The reserve exemplifies a typical ecological or environmental scenario found in Ecuador: Small patches of fragmented forest surrounded by agricultural lands. Six 600m transects were set up along the trail system of the reserve, this transect design was best to survey the reserve. In 16345 transect meters, and whilst moving in the reserve off transect, we encountered 14 individuals of *Bothrops asper* and 9 *Bothrops osbornei*. On encounter, spatial data was taken, and individuals were captured to be processed at the station. The focal species of this project are *Bothrops asper* and *Bothrops osbornei*. Presented are preliminary results and statistics of our ongoing investigation for the spatial ecology of *B. asper* and *B. osbornei*. There is a clear trend of *B. asper* being encountered almost exclusively on the floor, whilst *B. osbornei* is primarily encountered foraging at ground level and was only found resting in arboreal habitats. Even at larger sizes (117cm total body length) they were found at heights of 490cm suggesting that also bigger individuals utilize higher strata's of understory. Planned are another 4 field seasons at UPDC, considering conservation status of the locality and current knowledge on population estimates of neotropical pitvipers, we will (a) deliver the first population estimation for both species and (b) gain important information for future conservation efforts and snakebite mitigation work once more data has been collected.

ECOLOGY

Microhabitat Preferences of Herpetofauna in Coastal Wetland Ecosystems of the Biga Peninsula, Türkiye: A Preliminary Assessment

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Abstract

This study presents a preliminary assessment of the microhabitat preferences of herpetofaunal species in four coastal wetlands of the Biga Peninsula (Karabiga/Kocabaş River Delta, Güvemalan Hoyrat Lake, Tuzla Delta, and Kumkale Delta) in Türkiye. Between March and May 2025, Visual Encounter Surveys (VES) were conducted to identify amphibian and reptile species and record their associated microhabitat types. A total of 432 individuals belonging to 2 amphibian and 9 reptile species were documented. *Pelophylax bedriagae* was the most frequently recorded species (28.88%), while *Hyla orientalis* was the least common (1.19%). A total of 10 microhabitats preferred by species in coastal wetlands were identified, the Wetland habitat was the most intensively used across all sites, supporting 210 individuals (59.66% of all observations), while the Heterogeneous Agricultural Areas were used by only one individual. Chi-square analysis confirmed significant differences in microhabitat preferences among the wetlands (χ^2 (21) = 350.39, $p < 0.001$). Additionally, the calculated Shannon-Wiener diversity indices revealed that the Karabiga/Kocabaş River Delta had the highest overall species diversity, while the Kumkale Delta had the lowest. These findings emphasize the critical importance of maintaining permanent and semi-permanent aquatic habitats and structurally diverse wetland mosaics to sustain amphibian and reptile communities in coastal ecosystems.

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This study is part of the PhD thesis titled "Habitat Use Patterns and Niche Separation of Amphibian and Reptile Species in the Coastal Wetlands of the Biga Peninsula" funded by the Scientific Research Projects Commission of Canakkale Onsekiz Mart University (FDK-2025-5074).

CONSERVATION

A different future for two species of Balkan water frogs: bioclimatic niche divergence shapes species' responses to climate change

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Abstract

Amphibians are among the vertebrate groups that are most sensitive to global environmental changes, and this vulnerability is even greater in species with a limited range. In the southwestern Balkans, two endemic water frog species, *Pelophylax epeiroticus* and *P. shqipericus*, inhabit predominantly lowland wetlands that are increasingly threatened by climatic and anthropogenic pressures. Despite their geographical proximity and ecological similarities, our projections for 2050 and 2070 reveal markedly contrasting future trajectories for these species.

Pelophylax epeiroticus is predicted to undergo severe range contractions, with only a few isolated refugia remaining suitable under future climate scenarios, mostly in coastal regions and some islands. Conversely, *P. shqipericus* appears more resilient, with anticipated range shifts into higher elevations and persistence of suitable habitats across much of its current distribution. It seems that these differences are caused by distinct bioclimatic niches. Both species differ significantly along climatic gradients shaped by temperature, precipitation seasonality, with additional effect of elevation. These climatic preferences also correlate with differences in genetic vulnerability, as southern phylogenetic lineages of *P. epeiroticus* are especially prone to habitat loss, while phylogenetic lineages of *P. shqipericus* retain high stability over the whole species' range.

Our results suggest that even closely related and ecologically similar species may respond differently to global change due to subtle niche divergence and spatial

configuration of their habitats. The limited overlap between future refugia and existing protected areas, particularly for *P. epeiroticus*, highlights critical conservation gaps. Addressing these disparities will require adaptive strategies that incorporate climatic sensitivity, habitat quality, and the preservation of evolutionary lineages.

TAXONOMY

One species or two? How many species of *Trapelus* Cuvier 1817 occur in Morocco?

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Abstract

The genus *Trapelus* Cuvier, 1817, a member of the subfamily Agaminae, comprises at least 16 species, several of which are distributed in northern Africa, although the current taxonomic situation is still contentious. *Trapelus boehmei* Wagner, Melville, Wilms & Schmitz, 2011, was recently elevated from the *Trapelus mutabilis* complex to full species status, and is distributed across Mauritania, Morocco, and Algeria. A recent fieldguide indicates this as the only species of *Trapelus* found in Morocco, but noted that the identity of populations in the northeast of this country are uncertain, and may correspond to *Trapelus asperus* (Werner, 1893). This study presents a preliminary molecular analysis of diversity within specimens from across Morocco based on partial mitochondrial 16S ribosomal RNA gene sequences. Our analysis supports the hypothesis that two species of *Trapelus* occur in Morocco, *T. boehmei* across most of the country and *Trapelus* cf. *asperus* in the northeast. These species seem to be separated around the dry Moulouya river valley, a region that coincides with phylogeographic breaks in many other groups. Morphological assessments will be needed to confirm that the northeastern form corresponds to *T. asperus*, but our preliminary data highlights the continuing trend of identifying additional reptile species within Morocco, especially when molecular tools are applied.

ECOLOGY

Scales on rails? Modelling the distribution of Western Ghats King Cobras, *Ophiophagus kaalinga* Gowri Shankar et al., 2024, in Goa, India, reveals the potential effect of train journeys

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Abstract

We provide the first detailed documentation on the distribution and natural history of *Ophiophagus kaalinga* in Goa State, India, and its interesting apparent interaction with the railway system. The combination of rescue records, local reports, and historical data allowed us to document a total of 47 georeferenced localities where *O. kaalinga* has been found in the state, with 18 localities in North Goa District and 29 in South Goa District. These data inform our study of the Goa Gap, a biologically significant region without obvious physical characteristics, and assess the suitability of this area for king cobras. Using a series of climate and vegetation variables, we were able to model the distribution of the species in Goa. It is noteworthy that only five king cobra records fell outside the suitable habitat predicted by our model, and all of these errant records fall along busy railway corridors. Combined with recent reports of snakes on trains in India and of *O. kaalinga* in a rail yard, entirely unsuitable reptile habitats, we hypothesize that snakes, king cobras included, may inadvertently expand their ranges by taking the train.

CONSERVATION

Urban Wetlands and Forest Islands as Herpetofaunal Refugia: Amphibians and Reptiles of Belgrade's Protected Areas

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Abstract

Urban landscapes, particularly in large cities, impose strong pressures on native biodiversity. However, protected areas within metropolitan zones may serve as key refugia for sensitive faunal groups such as amphibians and reptiles. This study synthesizes findings from two field surveys conducted in 2022–2024 on the herpetofauna of two distinct protected areas in Belgrade, Serbia: Veliko Ratno Ostrvo (a river island at the confluence of the Sava and Danube rivers) and Obrenovački Zabran (a remnant of a forested floodplain on the city's periphery). Across both areas, a total of 17 amphibian and reptile species were recorded, several of which are under national or European protection. The sites host a mosaic of habitats including floodplain forests, ephemeral ponds, riverbanks, and anthropogenically altered landscapes, that support both habitat specialists and generalists. Despite their isolation and the surrounding urban infrastructure, both areas maintain viable populations of breeding and hibernating individuals, highlighting their ecological significance. The results emphasize the importance of integrative urban planning that prioritizes the conservation of relict wetland and forest ecosystems. These findings contribute to a broader understanding of how European and global metropolitan areas can play a role in herpetofaunal conservation through strategic management of protected green areas.

OTHER

A review of tail anomalies in lizards (Reptilia: Sauria) in Poland with additional records

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Abstract

Caudal autotomy is a defense mechanism in which an animal voluntarily loses its tail, resulting in the regrowth of it at the place of its loss. Once broken off from the caudal vertebra (in the “zone of weakness”), the tail stump heals quickly, and regeneration begins. Sometimes abnormal tail formations appear. These include tail furcations-split or multi-branched tails, sometimes even when the original tail is only partially detached. In taxa exhibiting tail regeneration, a new tail may develop even when the original one is not entirely detached, leaving the animal with a seemingly forked tail (caudal bifurcation). In recent years, two-tailed (bifid), double-tailed, and even poly-tailed (polyfid) lizards have been documented with an increasing frequency. This study reviews documented cases of tail anomalies in lizards from Poland, focusing on *Lacerta agilis* and *Zootoca vivipara*. A literature search used keywords related to tail anomalies and relevant species (*Z. vivipara*, *L. agilis*, *Anguis fragilis*, *A. colchica*, *Podarcis muralis*) in Polish, English, and French. Articles mentioning unrelated anomalies or species outside Poland were excluded. References within relevant articles were also examined. The search concluded on 01/08/2024, and online platforms like social media and iNaturalist were not used. Here, we present a review of the locations of previously documented anomalies in lizards in Poland, along with new additional records (*L. agilis* and *Z. vivipara*). Despite extensive fieldwork, lizard tail anomalies in Poland are rarely mentioned in scientific literature. While the consequences of tail loss are well-studied, the impact of abnormal regeneration remains poorly understood. Future research should focus on potential links to habitat types or geographic distribution, and examine the age and sex of individuals with such anomalies. Presenting known records and new cases, this review aims to support further study of this under-researched phenomenon.

CONSERVATION

LIFE-IP ForEst&FarmLand project restores 100 small water bodies in intensively used agricultural landscapes in Estonia**Riinu Rannap**

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Abstract

Intensive agriculture has led to the replacement of landscape mosaics with drained monocultural farmlands where pesticides and artificial fertilisers are heavily used. Due to the destruction of suitable habitat complexes, caused by the land use change, five out of the 11 species of amphibians found in Estonia are endangered. Two of such species, the common spadefoot toad (*Pelobates fuscus*) and the northern crested newt (*Triturus cristatus*) were selected as target species for restoration of small water bodies in protected areas (Natura 2000 sites) bordering with intensively used agricultural landscape. To secure the populations of these species, it is planned to restore 100 small water bodies in ten Natura 2000 areas in the vicinity of large agricultural areas between 2021 and 2027. This work is being done as part of the LIFE-IP project ForEst&FarmLand (LIFE18IPE/EE/000007). So far, 67 ponds have been restored in 10 protected areas and by 2025, target species had colonized 66 % of restored ponds for breeding in five protected sites. However, target species have not yet colonized the restored water bodies in the remaining five project areas.

CONSERVATION

The Trade–Invasion Link: Evaluating Exotic Turtle Markets and Propagule Pressure

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Abstract

Turtles are popular pets and are frequently introduced outside their native ranges. Although propagule pressure is key to invasion success, it is often hard to measure. Given the pet trade's major role in turtle introductions, it can serve as a useful proxy. While CITES regulates certain species, much of the global trade — including illegal sales — goes undocumented. This study investigates exotic pet turtle availability in both legal and illegal markets, analyses trade on social media, examines the relationship between propagule pressure and wild populations, and evaluates how well online markets reflect actual trade patterns, using Portugal as a case study. Data were gathered from pet stores, online platforms, and a Facebook group. We developed a Relative Market Pressure (RMP) index based on trade frequency across platforms as a proxy for propagule pressure. Our results showed marked differences in species composition across trade types, with online platforms — especially social media — driving much of the trade, including in banned species. Human population density strongly predicted exotic turtle presence in the wild. These findings highlight the need for monitoring both conventional and online trade. The RMP index offers a practical tool for assessing propagule pressure. We recommend stricter enforcement, adoption programs for unwanted turtles, and size-based sale restrictions to reduce releases and invasion risks.

ECOLOGY

Experimental insights on the behaviour and development of Near Eastern Fire salamanders (*Salamandra infraimmaculata*) indicate general strategies to cope with aquatic predators

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Abstract

Fishes are key predators of amphibian larvae with often devastating consequences for their abundance and survival. In Israel, the Near Eastern Fire salamander (*Salamandra infraimmaculata*) reaches the southern limit of the geographic distribution of the whole genus and must cope with xeric terrestrial habitats- and a scarcity of suitable sites for the deposition of larvae with permanent springs and streams are being rare. One such location is the Tel Dan National Park in the North of Israel, where salamander larvae coexist successfully with fishes in a system of larger and smaller streams. In three experiments we tested different anti-predator behaviour with salamanders from Tel Dan and other locations. We analysed whether gravid females avoid the presence of fish when depositing their larvae; if the presence of fish impacts larval development and if salamander larvae show active hiding behaviour when confronted with fish cues. While females from Tel Dan showed a bet-hedging strategy in site selection irrespective of fish presence, larval developmental rate was reduced in the presence of fish probably due to a lower feeding activity. Lastly, larvae from different sites displayed hiding behaviour in the presence of fish, irrespective whether they naturally coexist with fish or not. We infer from our results and existing studies that *S. infraimmaculata* shows general rather than site-specific adaptations to cope with the presence of fish. We conclude that the general adaptations are essential to sustain this species in a range of extreme habitats at the southern limit of the distribution.

CONSERVATION

Assessing populations' status and distribution of the endemic Ambrosi's Cave Salamander: first results

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Abstract

The Ambrosi's Cave Salamander (*Speleomantes ambrosii*), a Critically Endangered species endemic to northwestern Italy, faces significant potential threats from habitat restriction and the emerging pathogen *Batrachochytrium salamandrivorans* (Bsal). Thanks to the SEH Conservation Grant we started a project to assess the population status, distribution, and health of *S. ambrosii*. We searched for the species within and at the limit of its distribution range to find new sites of presence and confirm the uncertain ones. We also used the double observer (DO) method within a hierarchical framework to estimate population abundance and density in subterranean environments. In parallel, we also collected swabs for pathogen screening of Bsal which will be analyzed at the University of Genova.

We found that sites at the western and eastern boundaries, where the species presence had been erroneously reported in the past, were actually inhabited by the congeneric *S. strinatii*. Abundances and densities in subterranean habitat estimated with DO method, which confirmed to be highly cost-effective, were highly variable (from 7 to 207 estimated individuals), depending on the monitored cave. Our results show that the populations of *S. ambrosii* seem to be flourishing and in good condition, however the already limited distributional range would appear to be even less than estimated.

These first outcomes have already helped to enhance our knowledge of *S. ambrosii*'s status. Future monitoring will focus on continuing the search for new sites of presence, based on new remote sensing approaches and field research, and analyzing the swabs collected during the monitoring.

MORPHOLOGY

Long legged or wide-eared: Sexual size dimorphism and interspecific body size differences in Central European brown frogs (genus *Rana*)

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Abstract

Since 2010 Humboldt reloaded (<https://humboldt-reloaded.uni-hohenheim.de>), a teaching program based at the University of Hohenheim supports early undergraduate studies in a wide array of topics including systematic zoology. In 2025 four undergraduates examined three Central European brown frog taxa i.e., *Rana arvalis* (moor frogs), *R. dalmatina* (agile frogs) and *R. temporaria* (common frogs) for sexual size dimorphism and species-specific body size differences based on the holdings of the herpetological collection housed at the State Museum of Natural History Stuttgart (SMNS). Due to a lack of female agile frogs, female interspecific differences were not statistically tested.

Whereas male and female common and moor frogs lacked intersexual differences in tibia size, tympanum diameter and eye-tympanum distance relative to their body size, male common frogs and moor frogs had a larger eye diameter relative to their body size compared to their female counterparts. In an interspecific comparison of male frogs, typical species-specific differences were observed in relative size of morphological traits in proportion to overall body size (snout-vent-length in mm as a proxy). We finally could manifest marked interspecific differences with our data at hand.

BIOGEOGRAPHY

Who am I and if so how many? Distribution of non-native chelonians in Germany

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Abstract

Non-native species represent an increasing global concern. Yet, in many cases, the fundamental data needed to address this issue are lacking, as are opportunities to collect such data. A particularly illustrative example involves non-native terrapins. The Red-eared Slider (*Trachemys scripta*), originally native to parts of North America, has now established self-sustaining populations in many regions worldwide, continually expanding its range. All three subspecies are classified as invasive and are listed in relevant regulations. However, numerous other non-native terrapin species are also present but often go unrecognized.

Using quality-checked citizen science data from iNaturalist and other sources, we assessed the diversity and distribution of non-native terrapins in Germany for the first time. A total of 1,770 occurrence records revealed the presence of 14 non-native species: *Chelydra serpentina*, *Chrysemys dorsalis*, *Chrysemys picta*, *Graptemys ouachitensis*, *Graptemys pseudogeographica*, *Mauremys leprosa*, *Mauremys reevesii*, *Mauremys sinensis*, *Pelodiscus sinensis*, *Pseudemys concinna*, *Pseudemys nelsoni*, *Pseudemys peninsularis*, *Sternotherus odoratus*, and *Trachemys scripta*. Furthermore, we report many allochthonous individuals of the native *Emys orbicularis*. While, in addition to *T. scripta*, both *C. picta* and *G. pseudogeographica* have recently been shown to maintain reproducing populations in Germany, the distribution of all species shows a clear association with urban areas. The ecological impact of non-native terrapins in Germany remains unclear and warrants further investigation.

DISEASES

Impacts of *Batrachochytrium salamandrivorans* on Great Crested Newt (*Triturus cristatus*, LAURENTI, 1768) Populations: Evidence from a Multi-Year Capture–Recapture and qPCR Surveillance Program

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Abstract

Pathogenic fungi such as *Batrachochytrium dendrobatites* (Bd) and *B. salamandrivorans* (Bsal) cause considerable population declines in amphibians worldwide.

Chytridiomycosis caused by Bsal is usually fatal, especially in *Salamandra salamandra* (LINNAEUS, 1758). In other species, such as *Triturus cristatus*, mortality is not quite as high (75%) as in *S. salamandra*, but nevertheless, infection of *T. cristatus* with Bsal also leads to severe population declines.

The aim of our examinations was therefore to determine the occurrence of Bsal infestation of *T. cristatus* and its influence on population sizes and dynamics examining a former stable population in a quarry.

We used the capture/re-capture method for long-term monitoring of *T. cristatus* in several ponds of a quarry in North Rhine-Westphalia. Each individual caught was identified, counted, swabbed and implemented in our database by photographing the individual-specific belly pattern. The skin swabs subsequently taken using a standardized procedure were tested for Bsal using quantitative PCR.

Linking our molecular results with the photo data enabled an analysis of recoveries and the tracing of individual infection processes. After a stark decline of the population for several years, our actual data hint on an increase of animal numbers. We also recovered six Bsal-infected animals, some even after two years, still living.

Implementation of such long-term monitoring programs is fundamental for the detection of dispersal patterns and the targeted development of protective measures for the conservation of amphibian populations endangered by Bsal.

ETHOLOGY

Laterality in Toe-Tapping Behaviour of Green-and-Black Poison Dart Frogs (*Dendrobates auratus*)

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Abstract

Laterality, the preference for using one side of the body over the other, has been observed in various frog and toad species. Anurans with asynchronous locomotion (non-simultaneous limb movements; e.g. climbing & crawling), for example, show a higher percentage of left-/right-handed individuals than anurans with synchronous locomotion (simultaneous limb movements; e.g. swimming & hopping). While laterality has been studied widely during locomotion in anurans, its role during other behaviours, such as toe-tapping (the quick up and down movement of the middle toe of the hind feet), remains largely understudied. In this study, we investigated the toe-tapping rate of the left and right toes while both hind feet were visible in a captive colony of green-and-black poison dart frogs (*Dendrobates auratus*) in Frankfurt Zoo. Our aim is to see if individuals of this species tap their toes simultaneously or tend to tap more with either their left or right toes. We discuss our findings in the light of both, laterality in amphibians, and the search for a potential function of toe-tapping behaviours in anurans.

CONSERVATION

The data arrangers: Compilation, completion, and analysis of existing herpetological data in Austria

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Abstract

Effective conservation efforts — such as tracking amphibian decline, supporting reptile protection, fulfilling reporting obligations under Article 17 of the EU Habitats Directive, or updating national Red Lists—all depend on one essential resource: data.

In Austria, where only 21 amphibian and 15 reptile species are native, a large volume of occurrence data already exists. These records are stored across various sources, including institutional databases at the Natural History Museum Vienna (NHMW) and the Haus der Natur Salzburg, as well as public platforms like iNaturalist, Observation.org or Naturbeobachtung.at. However, this data landscape is fragmented, with inconsistent quality standards and accessibility. Furthermore, a considerable amount of valuable observation data remains unpublished and unarchived, and are at risk of being lost in drawers forever. To address this issue, the “Amphibians and Reptiles of Austria” project of the NHMW and cooperation partners is working to

compile and unify all existing herpetological records. This comprehensive dataset will allow for detailed analyses, providing the clearest picture yet of the distribution of Austria's herpetofauna and revealing data gaps. These gaps will then be addressed through targeted field surveys. Public involvement plays a vital role: citizen science initiatives such as the "Herpetorace" are designed to engage and mobilize the public to contribute new observations. This poster presents an overview of the project, challenges of the current data landscape, and the steps being taken to build a foundation for herpetological conservation in Austria.

The project is funded by the Biodiversity Fund of the Federal Ministry of Austria for Agriculture and Forestry, Climate and Environmental Protection, Regions and Water Management.

CONSERVATION

Frogs Without Borders: Phenology and Eradication Attempt of the Northernmost Invasive Population of African Clawed Frog (*Xenopus laevis*) in Europe

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Abstract

The African clawed frog (*Xenopus laevis*) has established invasive populations worldwide, posing a serious threat to local biodiversity. Despite extensive control efforts, documented successes are rare. We evaluated the effectiveness of fyke trapping and quicklime application in lentic systems to eradicate a novel population within a dense cross-border hydrographic network in Belgium and France. We also present phenology data for this northernmost population: reproduction began as early as April, and larvae metamorphosed within the same year, indicating potential for rapid population growth and colonisation. Monitoring with eDNA and fyke trapping shows that both eradication methods effectively reduce local densities. However, rapid recolonization highlights the need for long-term solutions. Moreover, the distribution of the African clawed frog population overlaps with that of the protected great crested newt (*Triturus cristatus*). We therefore launched a transboundary project with Wallonia and Northern France to implement a comprehensive, cross-border action plan focused on adaptive eradication to permanently remove this invasive *Xenopus laevis* population. The strategy moves from containment at the margins to core eradication. eDNA sampling plays a central role in guiding the project, serving as a detection tool for the spread of *X. laevis*, for estimating population sizes, for identifying sensitive areas via metabarcoding, and as a measure of the effectiveness of control actions. Next to the use of quicklime and intensive trapping, population control with native predatory fish is also considered. Where feasible, ponds will be permanently closed. Management decisions are made on a case-by-case basis, accounting for agricultural

activities and landowner consent. Effective stakeholder communication is hereby essential for continued access and project success.

ECOLOGY

Too hot to hatch: incubation temperature effect on egg survival and hatching time in *Podarcis siculus***Giulia Simbula**

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Abstract

Temperature plays a fundamental role in regulating reproductive functions and success in reptiles. In this taxon, elevated incubation temperatures typically accelerate embryonic growth, potentially conferring advantages such as shorter developmental periods. However, these thermal effects may trade off with increased mortality risk and altered phenotypes, as reduced body mass or elongation.

In this study, we investigated the effects of different incubation temperature on the embryos and hatchlings of the Italian wall lizard, *Podarcis siculus* from an invasive population in Lisbon (Portugal), originated from central Italy (Tuscany). Gravid females were captured and kept in captivity until oviposition. Embryo viability was assessed and, viable eggs were incubated at three different temperatures: 26, 29 and 32 °C in order to determine incubation time, embryo survival, and hatchling size. Overall, 61.8% of eggs were viable, while 38.2% were empty. Eggs incubated at 32°C hatched on average a week earlier than those at 29 °C, and 11 days before those at 26 °C. While no significant differences in egg volume emerged among groups, a higher incidence of developmental issues, including eggs mortality and hatchling malformations were observed at 32 °C. Newborns from the 29 °C group had the highest body size and mass. Once hydration was granted, incubation at 32 °C seemed to accelerate development but at the cost of decreasing hatchling survival and quality. Our results contribute to a broader understanding of how thermal conditions influence early developmental success in lacertid lizards, with potential implications for population dynamics in new environments and changing climates.

ECOLOGY

Investigating short-distance magnetic orientation in a Neotropical poison frog

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Abstract

Amphibians use a variety of cues for navigation, including visual, olfactory, and magnetic information. While magnetoreception has been demonstrated in several nocturnal and migratory species, its role in diurnal, territorial frogs remains unexplored. *Allobates femoralis* (Aromobatidae), a Neotropical poison frog, exhibits complex spatial behaviour, including long-distance tadpole transport, site fidelity and homing, making it an ideal model to test for magnetic orientation. This study investigates whether *A. femoralis* relies on geomagnetic cues to orient after displacement. Territorial males were captured and tested individually in the Nouragues Nature Reserve in French Guiana over two months in 2025. Each frog underwent two trials in a circular arena: one under the natural magnetic field and one under an altered field generated by a portable coil setup. The movements of the frogs were recorded with overhead cameras and analysed using circular statistics in R. Rainfall, ambient temperature and conspecific calling were also recorded to account for external influences on the behaviour. This project offers one of the first tests of magnetic orientation in a diurnal tropical amphibian, contributing new insight into the mechanisms underlying its spatial behaviour.

BIOGEOGRAPHY

Preliminary Survey of the Contact Zone of *Hyla intermedia perrini* and *Hyla arborea* in Slovenia

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Abstract

The contact zone between *Hyla arborea* and *Hyla intermedia perrini* along the Slovenian–Italian border remains insufficiently characterized. *H. arborea* is widespread in Slovenia, whereas *H. i. perrini* is distributed across the Po Plain and surrounding areas. In 2024, the presence of *H. i. perrini* in Slovenia was indisputably confirmed for the first time in Goriška Brda region. An adult tree frog found in the village of Neblo initially identified as *H. i. perrini* according to morphology was genetically confirmed using buccal swab samples and two molecular markers: mitochondrial cytochrome b and the nuclear rag-1. In 2025, we carried out a series of nocturnal surveys searching treefrog breeding sites in western Slovenia, with the focus on the putative contact zone, collecting skin or buccal swabs from 58 adults for molecular identification using the same markers. All individuals were photographed, and morphometric parameters were recorded on site. The newly obtained enhance our understanding of the distribution these taxa in Slovenia and provide novel insight into the extent and nature of the contact zone between *H. arborea* and *H. i. perrini*. Here, we present preliminary findings from this ongoing investigation.

OTHER

An automated acoustic classification model for monitoring Ukrainian amphibians using a deep neural network

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Abstract

Amphibians represent the world's most rapidly declining vertebrate group, demanding efficient and scalable monitoring methods for effective conservation. While Passive Acoustic Monitoring (PAM) with Autonomous Recording Units (ARUs) is a powerful tool, robust classification models for European amphibians remain scarce. This study addresses this gap by developing and validating a deep neural network, built upon the BirdNET framework, to identify the vocalizations of 11 common amphibian species in Ukraine: *Bombina bombina*, *Bombina variegata*, *Bufo bufo*, *Bufo viridis*, *Hyla orientalis*, *Pelobates fuscus*, *Pelophylax lessonae*, *Pelophylax ridibundus*, *Rana arvalis*, *Rana dalmatina* and *Rana temporaria*.

Our model was trained using a dataset of 15,213 3-second audio recordings. An initial validation on 2,235 clips demonstrated exceptional performance, achieving 100% accuracy for six species. Analysis of model calibration revealed that while most species showed a strong correlation between confidence and accuracy, classification was more challenging for others, such as *Pelobates fuscus* and *Rana dalmatina*.

For practical field validation, we analyzed 3,182 recordings from ARUs deployed in Nature Reserve "Roztochya". This confirmed the model's high reliability in identifying species like *Hyla orientalis*, *Rana arvalis*, and *Rana temporaria* under real-world conditions.

Overall, the model shows strong promise as a diagnostic tool for non-invasive amphibian monitoring. Its successful implementation has the potential to significantly enhance conservation efforts by enabling large-scale population assessments, facilitating the discovery of new habitats, and promoting wider engagement in amphibian research. To foster collaboration and widespread adoption, the finalized model will be published and made publicly available. Future work will focus on refining

model accuracy for more cryptic species and expanding its deployment across diverse ecosystems.

CONSERVATION

Preliminary results of the common toad *Bufo bufo* microchip-based monitoring in a semi-urban environment – a study from Poznań, Poland**Zyta Szadkowska**

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Amphibians, characterized by small dispersion means and a big attachment to breeding sites, can be especially negatively affected by habitat fragmentation. The common toad *Bufo bufo* is a species in decline due to a number of threats like climate change, destruction and alteration of habitats. Their migration routes are often disrupted by roads and railway tracks, resulting in direct mortality. This study has been conducted during spring migration to the breeding pond Staw Gołęciński in Poznań (52°25'48.7"N 16°53'17.5" E). The forest, which is likely a foraging area for the common toad population, is separated from the pond by train railway tracks and a road. The first migrating toads were found on the 9th of March, and the last on the 15th of April 2025. All of the captured animals have been weighed and measured. After the injection of microchips (type: HitagS-256/EM4305; size:1.25x7mm), they have been released at the capture site. During the study, 123 toads have been marked (M 117, F 6). All of the females have been captured in amplexus. Median SVL of males was 74.22 mm, and females 102.54 mm. Of 82 retraps, 24 individuals have been noted 2-4 times, with only one female retrap. Based on the location of recaptured toads, two possible ways of crossing the railway tracks can be distinguished: through a footbridge above the railway tracks and directly over the rail trail. Despite the road closure during spring migration, 7 dead individuals (one of them microchipped) have been found on the road and one on the rail trail. There were more victims when toads were migrating from the pond than to the pond. Although there was media coverage, many drivers choose to ignore the signaling as the road isn't physically blocked during the toads migration. It shows that a more active approach is needed, and just closing the road with appropriate road signs is not enough. In the next season, we plan to directly track selected individuals at the study site using telemetry.

CONSERVATION

Urban dwellers: species composition of amphibians in selected water bodies in Poznań, Poland**Natalia Szymańska**

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Abstract

Amphibians are an integral component of ecosystems and serve as sensitive indicators of environmental change. Monitoring their populations in urban areas provides valuable insights into the effects of habitat transformation on local biodiversity. The aim of this study was to investigate amphibian assemblages in 30 selected water bodies in the city of Poznań, Poland, and to assess the quality of their habitats with the Habitat Suitability Index (HSI).

Surveys were conducted from March to August 2024, with each site visited approximately twice per month. Species presence was confirmed through visual observations, acoustic detection, and dip-net sampling. HSI were evaluated in accordance with the national methodology for *Triturus cristatus* (Pabijan, 2010), itself based on Oldham et al. (2000).

In total, nine amphibian taxa were recorded (Anura: *Bombina bombina*, *Bufo bufo*, *Bufo viridis*, *Pelobates fuscus*, *Pelophylax kl. esculentus*, *Rana arvalis*, *Rana temporaria*; Caudata: *Lissotriton vulgaris*, *T. cristatus*). The most abundant taxon was the *P. kl. esculentus* complex, whereas the rarest species were *B. bombina* and *P. fuscus*. A 30-year comparative analysis revealed a decline in the mean number of species and a shift in species composition, although these changes were not statistically significant. Based on the HSI, only two water bodies were classified as habitats of favorable conservation status (FV), 16 were inadequate (U1), and 12 were in bad condition (U2). No significant correlation was found between HSI values and amphibian species richness ($p = 0.054$, $p = 0.776$), although *B. bombina* tended to occur in ponds with higher HSI scores.

Our findings highlight the need for long-term monitoring of amphibians in urban environments and for incorporating additional ecological factors influencing population dynamics in future studies. A better understanding of the determinants of species occurrence will enable the development of more effective conservation strategies.

CONSERVATION

Alternative concepts and common beliefs used for amphibians and reptiles in Çanakkale province**Gülçin Tan**

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Abstract

In this study, the current knowledge and common beliefs of people living in urban and rural areas in the districts of Çanakkale and the students of the Faculty of Arts and Sciences of Çanakkale Onsekiz Mart University, regarding amphibian and reptile species were investigated. The survey was conducted with a total of 126 people, 74 from the villages of the central district, 22 from the center of Biga, 10 from the center of Bozcaada, 10 from Çan and 10 from Gelibolu Kavakköy, and 157 students of the Faculty of Arts and Sciences of Çanakkale Onsekiz Mart University. The survey was conducted with a total of 282 people, 139 male and 143 female from the districts, villages and center of Çanakkale. In the survey, samples of a night frog (*Bufotes viridis*), a large green lizard (*Lacerta trilineata*), a sloth lizard (*Pseudopus apodus*) and a Caspian snake (*Dolichophis caspius*) taken from the collection of Çanakkale Onsekiz Mart University, Faculty of Arts and Sciences, Department of Biology, Zoology Museum were used to evaluate the alternative concepts and common beliefs that people use for frogs and reptiles. The results revealed that there is false information and beliefs about many harmless frog and reptile species.

EVOLUTION

The Relationship Between Host-Parasite Interactions and Diet Diversity

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Abstract

The Rock lizards of Georgia are a good model of host-parasite and environmental relationships using metabarcoding tools. Rock lizards exhibit two types of reproductive strategies: sexual reproduction and parthenogenesis. We plan to analyse the microbiome diversity in different species of rock lizards. According to the Deterministic hypothesis, frequent deleterious mutations act synergistically to cause a severe reduction in fitness compared to the previous mutation. According to this hypothesis, sexual reproduction provides an advantage over asexual reproduction that helps populations to eliminate deleterious mutations efficiently.

We are also investigating the "Red Queen" hypothesis, an evolutionary model in which recombination is important for species to adapt to their relationships with their parasites. If parasites are sexual breeders and the host is not, then parasites will adapt more quickly to maximise their impact on the host than the host will adapt to minimise the impact of the parasites. Therefore, studying the microbiota of asexual and sexual species of scaled reptiles in different environmental contexts can explain the "Red Queen" hypothesis. It is known that a parasite causes abiotic changes when it enters the host body, which can affect the host's diet. Research aims to improve our understanding of how microbiota diversity in reptiles is shaped by ecological factors such as climate or land cover, reproductive strategies, and taxonomic constraints. We plan to investigate how asexual organisms respond to parasites and adapt their genome without gene recombination, depending only on mutation. We will test the 'red queen' hypothesis and investigate the relationship between host-parasite dynamics and microbiota composition. This study provides valuable insights into the evolutionary and ecological processes that influence reptile fitness.

CONSERVATION

Extreme events: Quantifying the exposure of amphibian species to heat waves, cold spells, and droughts**Evan Twomey**

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Abstract

Globally, amphibians face severe threats, such as climate change and associated extreme events. Our goal was to quantify global amphibian exposure to three classes of extreme events: heat waves, cold spells, and droughts. We used the MERRA-2 extreme climate events data and the standardized precipitation-evapotranspiration index database (SPEI) to investigate where the incidence of these events has increased over the last 40 years. We used the IUCN database of global amphibian distributions to calculate the level of exposure to extreme events for each species, classifying species as exposed if their distribution has $\geq 50\%$ overlap with areas experiencing substantial increases of extreme events. We found that heat waves and droughts notably increased in Amazonia, Madagascar, and Europe. Among the three classes of events, exposure was highest to heat waves (40% of species exposed), followed by droughts (16% exposed). Exposure to different event classes was uneven with respect to geography and taxonomy, with some areas (e.g. Amazonia, Madagascar) and families (e.g. Mantellidae, Rhinodermatidae) having nearly 100% of constituent species classified as exposed to at least one event class. To assess whether exposure is associated with amphibian declines, we used logistic regression to analyze the relationship between extreme event exposure and status changes in the IUCN Red List, and found that exposure to heat waves and droughts is associated with status deteriorations during the past ca. 20 years. Our findings provide insight into amphibian biodiversity hotspots and taxonomic groups that may be particularly susceptible to extreme climate events, suggesting that these events play a causative role in ongoing declines. Understanding the aspects of species biology that influence susceptibility to extreme events, as well as interactions with other factors (e.g. disease), will be important for understanding the role of climate change in driving amphibian declines.

EVOLUTION

The evolution of sperm size and anisogamy in amphibians

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Abstract

Across the animal kingdom males benefit from producing numerous, low-cost sperm, while females invest more in costly eggs and offspring. It is known that in amphibians, larger eggs are associated with larger body size, and terrestrial egg-laying in frogs, and terrestrial or lotic egg-laying in salamanders. The predictors of sperm size are much less known. In this study, using data from 91 species of frogs (Anura) and 45 species of salamanders (Caudata), we used comparative analyses to assess predictors of sperm size in the two taxonomic orders. Phylogenetically controlled models indicate that salamanders have significantly larger sperm and a higher degree of anisogamy (gamete size bias based on sperm total length and egg diameter) than frogs, though egg size shows no significant order-specific differences. Interestingly, we find that larger sperm is associated with larger eggs in both orders. In salamanders with internal fertilization, the sperm has longer flagellum, while in frogs, testes mass (a proxy of sperm competition) and body mass are the main predictors of sperm head length. Our results indicate that sexual selection drives the evolution of sperm size, but different selection mechanisms act on head and flagellum length. In contrast, egg size evolved predominantly under environmental and life history selection, which often acts via offspring size. As a consequence the degree of anisogamy is hard to quantify, and it is a consequence of divergent selection processes on gamete size and shape.

ECOLOGY

Evolution of adhesive toepads and their ecological role in lizard habitat use and diversification

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Abstract

Adhesive toepads play a significant role from both evolutionary and ecological perspectives, as they enable adaptation to novel habitats, extreme weather conditions, and contributed to the adaptive radiation of the Squamata. The adhesive toepads of geckos, anoles, and skinks exhibit morphological diversity, as they have evolved through convergent and parallel evolution via different anatomical solutions. However, they are functionally homologous structures, as they all result in the same outcome and that is the effective adhesion. Previous research demonstrated that adhesive pads contributed significantly to the adaptation to arboreal lifestyle. Their role in rocky habitats are less obvious, however, it has been suggested that certain toepad types may have contributed to the occupation of karstic or dusty rock surfaces. In this study, we applied phylogenetic comparative methods on a comprehensive Squamata phylogeny to test how the presence and type of toepads influence habitat occupation in lizards. Our preliminary results confirm the association of toepads to arboreality, however, it also suggest that their did not facilitate the colonization of rocky habitats. Therefore, we propose that their key ecological innovation is adherence to smooth and less weight-bearing leaves and shoots, and they can be truly utilized by small-bodied species. In the next phase of our research, we plan to investigate the effects of different types of adhesive toepads, as well as their specific impact in toepad-bearing lizard clades.

EVOLUTION

Parental care intensity predicts vertical microbiome transmission potential in amphibians: a phylogenetic framework for future research**Dylan Vonhoff**

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Abstract

Amphibians are among the most threatened vertebrate groups, facing global declines largely driven by infectious diseases. Their skin microbiomes are increasingly studied for their role in disease resistance. Yet despite growing interest in amphibian microbiota, the potential for vertical microbial transmission via parental care remains underexplored. In contrast, such transmission is well documented in other vertebrate clades, including mammals, birds, reptiles, and insects. Here, we assess how different amphibian care strategies may facilitate vertical microbiome transmission and identify candidate taxa for future research.

To evaluate both data availability and analyse transmission potential, we first compiled information on microbiome presence, disease testing (Bd, Bsal, Ranavirus), and parental care for all 8,895 currently recognized amphibian species. This global dataset provides an overview of the current state of knowledge and reveals substantial gaps across lineages. From this broader set, we focused our analysis on 1,092 species for which both parental care data and 16S rRNA sequence information were available. Within the 328 represented genera, care strategies were reclassified by intensity and mapped onto a genus-level phylogenetic tree.

By comparing care intensities with known cases of vertical microbiome transmission in amphibians and other animals, we identified 150 species across 52 genera where care intensity aligns with conditions known to support vertical microbiome transmission. Our findings reveal phylogenetic clustering of intensive care strategies and suggest that vertical microbiome transmission in amphibians may be more widespread than currently recognized. Understanding where vertical microbiome transmission is most likely may inform future conservation and disease management strategies, while providing a roadmap for targeted experimental studies on host-microbiome inheritance in amphibians

EVOLUTION

How does hybridisation shape immune phenotypes?**Judit Vörös**

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Abstract

It is well known that across the tree of life, host species harbour different microbial communities, but the mechanisms governing assembly of these communities is poorly known. In our project we will investigate the role of host antimicrobial peptides (AMPs) in shaping the amphibian skin microbiome. Using bacterial isolates collected across a wild amphibian hybridisation zone transect (*Bombina bombina* and *Bombina variegata*), we will empirically determine the tolerance of skin commensal bacteria to host AMPs, and how this predicts microbiome community structure. In the first stage of this project we have characterised the skin microbiome and skin-secreted AMPs across two transects along *Bombina* hybrid zones – representing two mitochondrial lineages (Alpine and Carpathian) in Hungary (Bakony Mts and Mátra Mts). This spatial replication across two hybrid zones makes this system especially powerful in disentangling the role of host genetics and environment in shaping the skin microbiome. In June 2024 we sampled 60 *B.bombina*, 83 hybrids and 46 *B.variegata* across these two transects. Skin peptides were collected by bath exposure to Norepinephrine. Following Sep-pak purification, peptides were sequenced using LC-MS/MS on a Q-Exactive Plus (ThermoFisherScientific, UK) instrument in DDA acquisition mode. Bioinformatic processing was performed in PEAKs Studio XII. Skin microbiome was sampled by skin swab. We sequenced the V4 region of the 16S rRNA gene using a MiSeq system using a 500 cycle V2 chemistry kit (Illumina). The DADA2 pipeline was used for downstream bioinformatics processing of the raw data. Our preliminary results show that there are 5529 skin-secreted peptides produced in total across these two transects, including three known AMP families: Bombinin-like-

peptides, Feleucins and Proline-rich AMPs. We also found that there is co-variation in skin-secreted peptides and microbiome across this genetic gradient.

ECOLOGY

Mapping microhabitat use and habitat suitability for the endangered Moldavian Meadow Viper (*Vipera ursinii moldavica*) from the Danube Delta using remote sensing and species distribution modeling

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Abstract

Climate change, habitat loss, and illegal wildlife trade are among the main drivers of global biodiversity loss, severely impacting reptiles and amphibians. The Moldavian meadow viper (*Vipera ursinii moldavica*), one of Europe's most endangered reptiles, survives in isolated habitat patches under increasing anthropogenic pressure. Understanding how this species utilizes its environment at fine spatial scales is crucial for effective conservation planning. In this study, we integrated field observations on microhabitat use with remotely sensed imagery from satellite and small aircraft to

classify vegetation structure and environmental variables across a 62 ha site in the Danube Delta, Romania. Using location data from 75 individuals (29 females, 34 males, and 12 juveniles) collected during 2009–2010, we developed a habitat suitability model in Maxent based on a categorical microhabitat layer. This layer included seven habitat types: bare sand, *Juncus* spp., *Salicornia* patches, sparse vegetation on sand, wetland vegetation, open water, and wooded areas. Sampling effort covered the entire study area and was spatially random (Moran's $I = 0.012$, $z = 1.159$, $p = 0.246$). Our analyses show that *Vipera ursinii moldavica* preferentially selects areas dominated by *Juncus* spp. and sandy patches with sparse vegetation (GLM: $p = 0.039$). The Maxent model yielded a test AUC = 0.712, indicating good predictive performance. Predicted habitat suitability maps revealed several high-quality patches vital for population persistence. Preliminary analysis of sex-specific habitat use revealed largely overlapping preferences. This study is among the first to apply high-resolution microhabitat classification and species distribution modelling to an endangered reptile species. Our approach offers a practical and transferable workflow for informing habitat management and restoration strategies for *Vipera ursinii moldavica* and other species of conservation concern in fragmented landscapes.

ETHOLOGY

Preliminary data on the defensive behaviours of Nikolsky's Viper (*Vipera berus nikolskii*)

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Abstract

Predation is one of the most important drivers of natural selection and, consequently, evolution, acting on both predator and prey in the so-called “evolutionary arms race”. Reptiles have a multitude of natural predators, ranging from arthropods, amphibians and even other reptiles, all the way to carnivorous mammals and highly specialized raptor birds. This is why they had to evolve an impressive diversity of defensive strategies, both passive, like camouflage, aposematic colouration and mimicry, as well as active strategies, like fleeing, biting, defensive postures, musking, playing dead and many others. We explored the defensive responses of a venomous snake from Eastern and Southern Romania, the Nikolsky's Viper (*Vipera berus nikolskii* Vedmederja, Grubant & Rudajewa 1986) by simulating aerial attacks. The population most studied in this research which offered a greater sample size, is a polymorphic population with patterned (zigzag) individuals, as well as melanistic individuals and even some patternless grey ones (“concolor”), inhabiting forest edges, forest-meadows and railways. The snakes were caught by hand wearing thick welding gloves and held by the middle section of the body up in the air for 30 seconds, during which we recorded any behaviour displayed. Then, we processed the snakes, marked them by clipping the ventral scales and released them in the same spot from where they were captured. The scope of this study is to explore the correlations between various defensive behaviours and variables (colour morph, sex, age, previous wounds or captures, size, temperature, habitat etc.) and to analyse the prevalence of anti-predator responses throughout the studied population. None of the aforementioned correlations were statistically significant. Hissing was the most frequent defensive display (76.5%) Biting was scarcely employed (26.5%). Two rare behaviours were recorded for the first time in the Nikolsky's Viper: tail waving and hemipenis display.

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