

**Chasing the turtle: First assessment on spatial biology of endangered Spiny Hill turtle, *Heosemys spinosa* in Sarawak, Malaysia**

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Despite being on the Endangered list, the ecology of the Spiny Hill Turtle, *Heosemys spinosa* had remained unstudied. Yet, such fundamental knowledge as spatial ecology, behaviour and habitat use are critical for understanding the conservation and management needs of the species. We employed radio-telemetry to understand the spatial biology of the species, and six individuals were tracked up to 17 months. Data on home range, movement pattern and microhabitat preferences were recorded. Two important questions raised on the autecology of *Heosemys spinosa* relevant to its spatial ecology were as follows: 1) how does sexual variation, body size and environmental factors relate to habitat utilization and movement and 2) are there specific spatial zones of importance for life history and survival requirements. Home range for all individuals was estimates were 1.68-60 (mean 32.58 and 2.85) ha, in males and females, respectively, using 100% Multiple Convex Polygon (MCP). Although males showed larger mean home ranges compared to females (mean MCP: male 32.59 ha, female 2.85 ha), these differences are not statistically significant (Kruskall Wallis test:  $p > 0.05$ ), suggesting sex is not a reliable predictor of home range size. Carapace length, here a surrogate of size and mass, correlated with increased mobility ( $p < 0.05$ ), larger turtles obviously able to over greater distances, presumably for foraging, finding mates or new territory.

### Enter The Natrix: Ophidiomycosis and potential threats to European snakes

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Ophidiomycosis (formerly snake fungal disease), is an emerging infectious disease that causes skin lesions, ulcers, abnormal sloughing and in some cases mortality in snakes. The causative agent for ophidiomycosis is the fungus *Ophidiomyces ophiodiicola*. It is known from captive snakes, but until recently had not been found in the wild outside of North America. Following the first record of ophidiomycosis in Great Britain in 2017, one of the aims of this study focusses on investigating the prevalence of the disease and its potential impact on a population of barred grass snakes (*Natrix helvetica*) in England.

Swabbing and real-time PCR is being used to determine the presence of *O. ophiodiicola* and its relationship to skin lesions. The distribution and severity of skin lesions will be assessed in relation to body condition, age, demography and survival of individual snakes. The site was previously surveyed several times per week between 2015-2018 (by previous MSc students), using a combination of artificial cover object searches and visual encounter surveys.

Photos of the captured individuals are being used for mark-recapture analysis in order to appraise individual survival both within years and between years, as well as estimating the overall population at the site. This project integrates population monitoring with targeted disease surveillance. These two approaches are complimentary to, and able to inform, one another, with the hope of understanding how ophidiomycosis affects wild European snakes.

**Identifying genetic lineages through shape: an example in a cosmopolitan marine turtle species using geometric morphometrics**

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The green turtle (*Chelonia mydas*) is a globally distributed marine species whose evolutionary history has been molded by geological events and oceanographic and climate changes. Divergence between Atlantic and Pacific clades has been associated with the uplift of the Panama Isthmus, and inside the Pacific region, a biogeographic barrier located west of Hawaii has restricted the gene flow between Central/Eastern and Western Pacific populations. We investigated the carapace shape of *C. mydas* from individuals of Atlantic, Eastern Pacific, and Western Pacific genetic lineages using geometric morphometrics to evaluate congruence between external morphology and species' phylogeography. Furthermore, we assessed the variation of carapace shape according to foraging grounds. Three morphologically distinctive groups were observed which aligned with predictions based on the species' lineages, suggesting a strong genetic influence on carapace shape. Based on the relationship between this trait and genetic lineages, we propose the existence of at least three distinct morphotypes of *C. mydas*. Well-defined groups in some foraging grounds (Galapagos, Costa Rica and New Zealand) may suggest that ecological or environmental conditions in these sites could also be influencing carapace shape in *C. mydas*. Geometric morphometrics is a suitable tool to discriminate between genetic lineages in this cosmopolitan marine species. Consequently, this study opens new possibilities to explore and test ecological and evolutionary hypotheses in species with wide morphological variation and broad geographic distribution range.

**A metabarcoding study of diet and parasites in reticulated pythons (*Malayopython reticulatus*) in Singapore**

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The reticulated python, *Malayopython reticulatus*, is the largest terrestrial apex predator in Singapore. It preys on a wide variety of animals and is a definitive host for several gut parasites with small mammal-python life cycles. Previous studies of python diet and parasites used morphological analyses of fecal matter and gut dissections. This study utilised high-throughput metabarcoding to simultaneously characterise diet and gut parasites from fecal matter. From 91 fecal samples analysed, 14 prey and 75 nematode Molecular Operational Taxonomic Units (MOTUs) were detected using the COI gene. Pythons in Singapore largely feed on introduced commensal rat species. Other animals such as Sunda pangolin and barred buttonquail were also detected. A high diversity of nematodes were detected in the fecal matter. Of the 75 nematode MOTUs, only nine were identifiable against the COI barcode database. Most of these identifiable nematodes indicated a rodent diet. One python gut parasite, *Ophidascaris infundibulicola* was found to be prevalent in python fecal matter. The high number of unidentifiable nematode MOTUs highlights the importance of gut parasite surveys and barcoding. A fragment of the 18S gene was also sequenced from 18 fecal samples, revealing the presence of *Sarcocystis singaporensis* and *S. zamani* in pythons in Singapore. *Sarcocystis nesbitti*, a known human pathogen, was not detected in any samples. Metabarcoding has higher taxonomic resolution than morphology-based diet and parasite assessments. However, forensic prey DNA is highly degraded by the python digestion and is difficult to detect. Future studies should utilize both molecular and morphological techniques in tandem.

### Evolutionary consequences of isolation by environment in two distinct fire salamander ecomorphs

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

## Uncovering diversity of karst dwelling geckos in Thailand: Insights from morphological and molecular evidence

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Karst ecosystem harbors high levels of endemic species of plants, vertebrates and invertebrates that adapted to this particular environment. Among vertebrates, a large number of reptile taxa have been described from karst ecosystem, including geckos. The new discoveries of karst dwelling geckos have been increasing over the past decade, including genera *Cnemaspis* and *Gekko*. A combination of morphological, and molecular analyses was used to uncover the diversity of karst dwelling geckos in Thailand. The phylogenetic relationships of Rock gecko, *Cnemaspis* in the *siamensis* and *chanthaburiensis* groups were investigated based on mitochondrial DNA (NADH dehydrogenase subunit 2). The morphological and molecular data provided the evidence for describing four new species of *Cnemaspis* from karst ecosystem of the Thai portion of the Thai-Malay Peninsula between 2017–2019, indicating that unrecognized diversity might be hidden within the complex of morphologically similar species. Comparing to *Cnemaspis*, only a few species of *Gekko* were reported from karst ecosystem in Thailand. A new karst dwelling gecko, *Gekko flavimaritus* in the *G. petricolus* group was found from karst in central Thailand and its description brings a total number of *Gekko* in Thailand to eight. In addition, our phylogenetic analysis revealed the position of *G. lauhachindai* and verified its morphological assignment to the *G. petricolus* group. Additional field surveys in karst regions are needed explore the true diversity of karst dwelling geckos in Thailand which could be used for biogeographic studies and conservation implication.

### Evolution of sex chromosomes in snakes: a molecular-cytogenetic perspective

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For over 50 years, it was believed that all snake lineages share homologous ZZ/ZW sex chromosomes: homomorphic and poorly differentiated in “henophidian” snakes (a group consisting mainly of boas and pythons), but heteromorphic and well differentiated in caenophidian snakes. Recent studies revealed that homologous and differentiated sex chromosomes are indeed shared among all families of caenophidian snakes, but poorly differentiated sex chromosomes (both XX/XY and ZZ/ZW) were recently reported in four species of “henophidian” snakes in previous studies. Since the evolution of sex chromosomes in snakes is apparently more complex than previously thought, we examined 13 species of caenophidian and 12 species of “henophidian” snakes by classical and molecular cytogenetic methods. In caenophidian snakes, we proceeded with comparative study of the evolutionary dynamics of the repetitive content of the W chromosomes. Our results demonstrated a striking variability in the morphology and the repetitive content of the W chromosomes even between closely-related species, which is in contrast to the homology and long-term stability of the gene content of their Z chromosomes. In “henophidian” snakes, we applied cytogenetic methods, aiming to reveal their sex chromosome constitution. We applied Comparative Genome Hybridization (CGH) to uncover sex-specific regions of the genome and Fluorescence in situ hybridization (FISH) to test the distribution of repetitive elements frequently accumulated in differentiated sex chromosomes in vertebrates. Our results show that all examined “henophidian” species don’t possess sex-specific differences in their genomes and the evolution of sex chromosomes in “henophidian” snakes should be further explored with high-throughput methodologies.

### **The toxins beyond typical venom glands: evidences for venom production in other snake oral glands**

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Venom is generally assumed to be produced exclusively by serous glands in the upper jaw of snakes and delivered into preys through specialized front-fangs. However, rear-fanged snakes are recognized to produce venom in their Duvernoy's gland. Moreover, some evidences now point out the possibility of venom production even in the lower jaw glands in some clades. In order to better understand these systems and to check for the presence of toxins produced in these glands, we are conducting a large-scale comparative investigation of oral glands based on gene expression analysis and proteomic characterization, complemented by morphoanatomical examination. Transcriptomic analysis using paired-end Illumina sequencing revealed that typical venom toxins are generally expressed in the homologous Duvernoy's gland of Dipsadinae snakes, however they also produce novel exclusive toxin types such as Matrix Metalloproteinases. In goo-eater snakes (Dipsadini tribe), a peculiar infralabial protein-secreting system with a duct ending at the tip of the mouth was observed and we now show that it expresses low levels of toxin-like transcripts. In the front-fanged coral-snakes (*Micrurus spp*, Elapidae) we also observed the expression of toxin coding transcripts in the complex of mucous and serous glands in the lower jaw, which we demonstrated to include a highly developed rictal gland. Our results highlight the functional plasticity of snake oral glands, which should involve mechanisms of activation of specific venom genes to possibly turn on the venom production in homologous and non-homologous tissues.



**Oviposition site preference by Jeju salamanders (*Hynobius quelpaertensis*) on Jeju Island, Korea****Bae Y<sup>1,2</sup>**, Borzée A<sup>1</sup>, Heo J<sup>1,3</sup>, Kim J<sup>4</sup>, Jang Y<sup>1</sup><sup>1</sup>Laboratory of Animal Communication, Division Of Ecoscience, Ewha Womans University, <sup>2</sup>Department of Life Science, Hallym University, <sup>3</sup>Department of Biology, Jeju National University, <sup>4</sup>Department of Life Science, Yeungnam University

Most oviparous animals must choose adequate oviposition sites for their offspring to survive and grow. In amphibians, water quality is generally the most important factor for breeding, with pH, humidity, flow, depth and water temperature being among the most important variables influencing oviposition site selection. *Hynobius* salamanders generally lay eggs in slow flowing streams and small lentic water bodies such as puddles and fallow rice paddies. However, *Hynobius quelpaertensis* displays different patterns of oviposition site selection on Jeju Island in the Republic of Korea, where fresh water is usually restricted to small volcanic craters made of permeable basalts. For this study, we conducted surveys at 47 randomly selected sites in natural habitats between January and March 2019 and measured water depth, waterbody length, pH, humidity, air temperature, water temperature, conductivity and egg presence or absence. Our results show that *H. quelpaertensis* on Jeju Island prefers large and deep waterbodies for oviposition sites, while the other variables recorded were not significant. The preference expressed by the species here is divergent from the one expressed by most other *Hynobius* species in North East Asia, which generally select small and shallow wetlands. A potential explanation is that permeable basalts are likely to result in the oviposition sites being drained before the completion of tadpole development, and therefore sites with deep water are less likely to drain before development completion. We recommend further study on the variation of oviposition site preference for *H. quelpaertensis* on the Korean mainland and Jeju Island.

**Isothermal amplification enables molecular based field diagnostics of pathogens: A case study in *Batrachochytrium dendrobatidis***

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The use of molecular techniques to detect pathogen presence in hosts or environment has been integral part of epidemiological research. However, to date, there has only been limited methodology that can be adequately implemented in field-based studies. This study focused on the chytrid fungus *Batrachochytrium dendrobatidis* presents our experience with the use of isothermal amplification as a method allowing fast and sensitive detection of the pathogen.

The whole procedure including swab sampling, DNA extraction and analysis was designed and tested to be used both within a laboratory environment and field conditions. The portable device we used, Genie II. (OptiGene Ltd., UK), collects data on amplification of DNA and the anneal temperature of the product is measured at the end of each run. Both measurements are based on real time fluorescence detection. Data obtained by LAMP assay are closely correlated with results provided by universally accepted Boyle *et al.* 2004 qPCR and thus allow approximate quantification. The assay has been commercially available for several years now, and it has been used in Czech Republic in conservation projects, providing data on pathogen presence and prevalence dynamic in a complex amphibian community.

Testing amphibians on site in less than hour time from swab to result, can be used to identify positive individuals for intervention, pathogen collection or fast decision making in suspected cases of disease outbreak. Despite its high accuracy and faster amplification rate LAMP relative to qPCR, is comparatively unknown by most researchers.

## Differing Colonization Rates of Restored Wetlands by Amphibians on an Agricultural Landscape

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After 150 years of agriculture in the USA, the expanse of prairie potholes has been converted into hectares of row-crops. Collaborative efforts of federal, state, local government agencies, and private landowners restored >5000 ha of wetlands in northern Iowa. We document rates of colonization by amphibians among restored wetlands and factors contributing to them. Our study species included three anurans – Northern Chorus Frog (*Pseudacris maculata*), Northern Leopard Frog (*Lithobates pipiens*), American Toad (*Anaxyrus americanus*) – and Eastern Tiger Salamander (*Ambystoma t. tigrinum*). We used multiple surveys (2008-2011 for anurans and 2014-2016 for salamanders) and program MARK to estimate occupancy ( $\psi$ ) among 67 ponds scattered among 28 restored wetlands of varied size, age, and distance from other wetlands. Occupancy rates ( $\pm 95\%CI$ ) after 3 years for Chorus frogs, Leopard frogs, toads, and salamanders were  $100\pm 0\%$ ,  $70\pm 12\%$ ,  $79\pm 11\%$ , and  $74\pm 21\%$ , respectively. Chorus frogs were present and calling in restored wetlands the year following restoration, regardless of distance to the nearest occupied wetland. Colonization by toads and salamanders required 1-2 years, and Leopard frogs required up to 3 years. Radio-telemetry tracking showed that toads would move through row-crops extensively and salamanders moderately. Leopard frogs rarely used row-crops, using instead primarily roadside ditches and fence rows. Biophysical models recorded that the physiological cost of row-crops was low for only 2-3 months of the year. Differing tolerances to dehydration, size of the animal, and differences in over-wintering requirements explained these different rates of colonization of restored wetlands.

**A new species of dwarf burrowing skink from the Western Cape, South Africa****Bates M<sup>1</sup>**<sup>1</sup>*National Museum*

The mostly southern African scincid genus *Scelotes* consists of 21–24 species of small, attenuate, fossorial lizards exhibiting varying degrees of limb loss. Several species are found only in coastal areas, and a few, like *S. gronovii*, are restricted to coastal dunes and associated areas in the Western Cape Province of South Africa. Morphologically, *S. gronovii* is easily distinguished from others in the genus by its reduced monodactyl hindlimbs and the absence of forelimbs. However, a recent molecular phylogeny using three mitochondrial and two nuclear gene markers indicated that this species consisted of two distinct genetic lineages, one in the Elands Bay area (north of the Berg River) and the other in the vicinity of Langebaan. Examination of specimens used for this molecular analysis indicated that the two lineages, apparently separated by the Berg River valley, are easily distinguished by the relative length of the hindlimbs and differing dorsal colour patterns. Additional museum material was also easily assigned to either of the two forms using these characters. The population north of the Berg River is described as a new species. Although the IUCN Red List conservation status of *S. gronovii* was recently downgraded from Near Threatened to Least Concern, the small geographical ranges of the two species as currently defined is of concern and requires re-assessment.

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### Assessing Sublethal Effects of Pathogen Infection in Green Frogs, *Lithobates clamitans*

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Global decline and extinction of amphibian populations are attributed in large part to emerging infectious diseases caused by pathogens including the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), and ranavirus. Some species, such as the Green Frog, *Lithobates clamitans*, tolerate infection and serve as models to better understand disease vulnerability and resistance. Resistance may be related to extrinsic factors of a frog's environment, or may be an inherent to the frog's innate immune system. Proteins produced in frog skin secretions and metabolites produced by beneficial bacteria in the skin microbiome offer intrinsic protection against pathogens and are extremely variable among frog species. Disease resistant species, however, may be vulnerable to sublethal effects of pathogen infection that may be exacerbated by environmental stressors. We are monitoring populations of male *L. clamitans* in three locations in Maine that vary in habitat quality and land use. Isolated island populations are not infected with either pathogen and have relatively low levels of innate immune defense. Inland populations from areas with variable degrees of residential development and agricultural activity test positive for both pathogens and harbor *Bd*-inhibitory skin bacteria and *Bd*-inhibitory skin secretions. We are evaluating behavioral, morphological, and immunological characters, including calling activity, breeding coloration, and symmetry, to determine if males in these populations exhibit signs of stress that could compromise immune defense or reproductive fitness. Preliminary results suggest that populations differ in these characters among the three locations, but there is little evidence of significant differences in these features between infected and uninfected males.

**First records of *Batrachochytrium dendrobatidis* in the endangered mountain frog (*Philoria kundagungan*) from eastern Australia**

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<sup>1</sup>*Southern Cross University*, <sup>2</sup>*Australian Museum*

The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has been implicated in the extinction of seven species in Australia to date. However, the full extent of its impact is still unknown due to data deficiency. In this study, we investigated the occurrence of *Bd* in the endangered Mountain Frog (*Philoria kundagungan*), a poorly known, range restricted species endemic to the headwater streams of cool temperate rainforests on the New South Wales and Queensland border in eastern Australia. We detected *Bd* via quantitative PCR on skin swabs from *P. kundagungan* across its full geographic range (an area of <300km<sup>2</sup>) including remote wilderness areas, rarely visited by humans. Overall infection prevalence was 47.3% of 75 individuals sampled, and 57.9% of 19 headwater streams sampled contained *Bd* infected individuals. Infection prevalence varied among headwater streams, but was not associated with elevation, snout-vent length, mass or sex of the examined frogs. Pathogen load ranged from 0 to 12435 zoospores, and mean infection intensity was highest in the south corresponding with higher rainfall and lower temperatures. Although no individuals were observed with clinical signs of chytridiomycosis, this was unsurprising given the typically short duration of clinical chytridiomycosis, and the cryptic nature of the species which necessitates detection via male advertisement calls. Hence, the impact of *Bd* on this species is still uncertain. As inhabitants of headwater streams, Mountain Frogs may act as a source of infection for amphibians downstream. Our results confirm that *Bd* is common in headwater streams even in highly protected and remote areas.

### **Adaptive strategies of amphibians with regard to wintering in the north of polirctic**

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Only 5 species of amphibians, out of almost 200 ones of the Palaearctic fauna, inhabit the Arctic Circle. Their adaptation to the critical factors of the winter environment are different.

*Salamandrella keiserlingii* survives -55°C, and does not depend on the wintering temperature and occupies a huge areal (Berman et al., 2016).

*Rana arvalis* winters near the soil surface, freezes, but does not survive below -16°C. This temperature is an invincible obstacle during penetration to the area of continuous permafrost.

*R. temporaria* and *R. amurensis* winter in water, since they do not tolerate below -2...-2.5°C (Berman et al., 2017). An obstacle for distribution *R. temporaria* over the Urals is the extremely low oxygen content in the waters of the Ob basin in winter – it is intolerant to hypoxia and needs over 3mg/l oxygen concentration.

*R. amurensis* winters in waterbodies almost deprived of oxygen. Such conditions are characteristic of many reservoirs of northern Asia, and *R. amurensis* penetrated up to 71°N through them. The ability of a frog to survive anoxia for months was confirmed experimentally (Berman et al., 2019).

*Bufo bufo* does not tolerate freezing, but survives prolonged (more than 1 month) supercooling (-1°C) and can stay in water with an oxygen concentration of 3-4mg/l, i.e. can only winter deep in non-freezing soils or in non suffocation waterbodies. Thus, *S. keyserlingii* and *R. amurensis* have unique adaptive capabilities for the amphibian class and are widespread (unlike *R. temporaria* and *B. bufo*) in the coldest regions of the Palearctic.

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**Genetically and Morphologically Distinct Clades within the Tailed Frog (*Ascaphus truei* and *A. montanus*)**

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The Pacific Northwest of North America is home to the endemic, basal sister lineage of leiopelmatid frogs, the genus *Ascaphus*. The two species in this genus are adapted to cold, forested headwater streams and glacial meltwater streams. *A. truei* range from British Columbia south to northern California, and *A. montanus* occur inland in the northern Rocky Mountains. We compared differences based on published results and our new analyses. We sampled mitochondrial genes CytB and ND2 to ascertain range-wide genetic diversity of both species. Our results confirm the separation of interior and coastal species. Further, *A. truei* possesses previously undescribed marked subdivisions, suggesting a dynamic population history. We attribute these differences to repeated range expansion and contraction in areas most affected by glaciation, and southward, isolation and expansion. Some clades within *A. truei* are highly distinct. Besides genetic analyses, we also investigated morphological differences in the larval stage. Our tooth row data from *Ascaphus* larvae support our genetically-identified clades. Prior studies by others showed marked morphological differences in adults. In both Canada and the United States, *A. truei* is considered as a species of Special Concern. Current evidence suggests that populations in degraded habitats have reduced genetic diversity owing to lower effective population sizes and contractions of population size. We predict that there will be increased isolation and loss of populations due to habitat fragmentation and climate change. Lastly, these are species that also merit enhanced protection and management because they are evolutionary distinct taxa.



### **Production of viable *Ambystoma mexicanum* embryos from cryopreserved spermatozoa**

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Mexico is home to 17 *Ambystoma* species, the highest diversity in the world but alarming rates of decline continue making ex situ management of these species of the utmost priority. Cryopreservation provides a spatially and cost-effective method of preserving genetic diversity of endangered wild populations. In this study we cryopreserved sperm, collected from *Ambystoma mexicanum* males artificially stimulated with either 500 IU or 1,000 IU human chorionic gonadotropin (hCG). Sperm was frozen in solutions containing 5% sucrose, 1% DMSO, SAR and FBS individually or in combination. Three different sperm freezing techniques were tested, straws containing sperm were: 1) were plunged directly into liquid nitrogen and frozen at a rate of ~300° C /minute, 2) placed in vapour, 20 cm above the LN2 surface and cooled for 15 min before plunging into LN2, and 3) slow cooled at 4° C for 5 min, transferred to -20° C for 15 min and then plunged into LN2. Straws were thawed by, 1) placement in a water bath at 25° C for 5 min and, 2) by placement in a 40° C water bath for 3.3 min. Results indicated a post-thaw viability of 61.30 % ± 14.04 % (mean ±1 SD), with 1% DMSO resulting in higher (P<0.05) percentage rate of sperm recovery. No significant effect of cooling treatment or thawing was observed (P>0.05). Subsequent in vitro fertilisation with frozen sperm resulted in the following cleavage rates of, 80% in 1% DMSO/SAR, 70% in 1% DMSO/SAR/FBS, 60% in 5% sucrose and 56% in SAR.

**Brumation, an essential adaptation for growth and fitness in the sub-adult mountain yellow-legged frogs, *Rana muscosa***

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Mountain yellow-legged frogs (MYLF) occupy high elevations in the mountains of Southern California, where in most of the extant range brumation is an obligate response to cold temperatures and frozen streams. This adaptation provides protection from harsh weather and minimizes metabolic demand when food resources are scarce. However, in captivity, brumation is often avoided due to concerns regarding slow growth rates (affecting the onset of sexual maturation), compromised immunity and increase morbidity. Arguments countering these ideas take the philosophical stance that husbandry that mimics nature, where possible; will better address animal health, welfare and reproduction. Here, we evaluated the effects of brumating juvenile southern 1st-year MYLFs, in a conservation breeding and reintroduction program. Artificial brumation was achieved by steadily lowering water temperatures to ~5 °C and maintaining juveniles at this temperature for 1 or 3-months. We compared growth and post-release outcomes among the 1-month, 3-month brumated and controls (non-brumated) frogs. The results of this study indicate that 1) MYLF juvenile's brumated in captivity grow to sizes and weights similar to controls within 3 to 4 months following brumation; 2) Brumated frogs have faster growth rates than the control conspecifics that grow steadily over time. Moreover, post-release fitness of recaptured frogs was higher for brumated compared to non-brumated animals in the first 6 months post reintroduction. These results highlight the fitness benefits of brumating high alpine species such as MYLF and provide supporting evidence that it is important to replicate key environmental conditions present in nature in conservation breeding programs.

### Prey choice, feeding performance and ecological niche modeling of *Pareas* species in Taiwan

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The members of Pareidae (Squamata: Serpentes) are regarded as dietary specialists of terrestrial pulmonates. Most pareid snakes have asymmetric jaws, with their teeth on the right side more than those on the left side. Previous studies suggest that the evolution of asymmetric jaws in Pareidae is an adaptation to feed on dextral snails. However, the level of teeth asymmetry in Pareidae varies among species. For example, *Asthenodipsas malaccanus* has almost symmetric jaws and feeds mainly on slugs, whereas *P. iwasakii* is highly asymmetric. Thus, the extent of teeth asymmetry may reflect the feeding preference of Pareidae snakes. *Pareas* species in Taiwan (*P. atayal*, *P. komaii*, and *P. formosensis*) have different levels of teeth asymmetry, which make them suitable to test the hypothesis. Behavioral experiments show that most individuals of *P. atayal* and *P. komaii* predate on both slug and snail, yet *P. formosensis* was never recorded to prey on a snail. In terms of feeding performance, the feeding time on snail is significantly shorter in *P. atayal* than in *komaii*, but the two species do not differ in the feeding time on slug. Ecological niche modeling shows that the distribution of the snail (*Nesiohelix swinhoei*), the distribution of the slug (*Meghimatium fruhstorferi*), and altitude contribute the most to *P. atayal*, *P. formosensis* and *P. komaii* respectively. In conclusion, our results indicate that *P. formosensis* may specialize on slugs, and that *P. atayal* may perform better in handling snails than *P. komaii* does.

**Understand variation in advertisement call attributes in spring peepers (*Pseudacris crucifer*)**

**Chen Y<sup>1</sup>**, Loughheed S<sup>1</sup>

<sup>1</sup>*Queen's University*

The mate recognition system is the foundation of biological speciation in anurans. Sexual selection has been suggested to cause male call evolution. It is generally assumed that a significant fraction of variation in male call has an underlying genetic basis and that there is some link between call attributes and fitness, yet there are largely unproved assertions with many other factors contributing to diversity of calls within a male chorus. Anurans advertisement calls are important in studies in sexual selection, speciation as well as phenological studies. In all of these, understanding heritability is a prerequisite to understanding evolutionary patterns and potential responses to selection. However, few studies have examined the heritability of advertisement calls in anurans. In general, we know that key aspects of anuran calls, including temporal and spatial attributes, vary with morphology such as body size, ontogeny such as age, abiotic factors such as temperature, as well as biotic interactions like inter-male competition. In this study, we hope to quantify the genetic-basis of anuran call variation within a calling assemblage of a temperate treefrog, *Pseudacris crucifer*, and disentangle the relative contributions of different factors: body size, age, calling temperature, and genetics. We use single nucleotide polymorphisms (SNPs) to estimate the relatedness of calling males, and use animal model to incorporate influential factors. By investigating the genetic basis as well as environmental correlates of advertisement call attributes, we will gain a better understanding of call evolution and the potential for sexual selection to drive divergence.

### Camouflage and active pattern of *Takydromus* lizards at the twilight moment under avian attack risks

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*Takydromus* lizards (Lacertidae), the East Asian grass lizards, are characterized for their small body size, slender body shape, elongated tail, and the specialized behavior to perch on grass surface as camouflage at night. This behavior might be an adaptation to avoid predation from nocturnal rodents and shrews on the ground. However, they have to leave the grass surface as soon as possible after the daybreak moment to prevent avian predation from the top. Therefore, we aimed to investigate this predator-prey relationship by studying the effectiveness of their camouflage at the twilight period, as well as the visual diagnostic ability of birds at the same moment. We used digital cameras to record timing and behavior of the green-spotted grass lizard (*Takydromus viridipunctatus*) around the daybreak moment. The results indicated that they open their eyes at  $24.71 \pm 8.36$  minutes (mean  $\pm$  SD) before sunrise, and start the first move at  $10.45 \pm 7.02$  minutes (mean  $\pm$  SD) before sunrise. By using spectrometer and power meter, we further recorded the luminance at these two moments, simulated the contrast between lizards and grass background under this circumstance, and evaluated the visual diagnostic ability of the Cattle Egret (*Bubulcus ibis*) which has been proved to be the major predator of *T. viridipunctatus*. Our results represent a congruent timing of lizard movement and the critical effectiveness of camouflage; the underlying mechanism is that the lizards might have to move before the egrets are able to recognize their color and shape when perching on the grass.

## Preying upon a pathogen: The effects of species interactions on chytrid fungus

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Due to the amphibian fungal parasite chytridiomycosis, the past half-century has seen the global decline of at least 501 amphibian species with 90 presumed extinctions. With the risk of potential outbreaks in new areas, chytrid fungus (*Batrachochytrium dendrobatidis*) poses an ongoing threat to amphibians. While substantial research has focused on mitigating this disease, there are currently no feasible management solutions. Biological interactions can play a key role in mitigating chytrid fungus but have not been adequately assessed. The aquatic crustacean *Daphnia*, for example, can reduce amphibian infection rates by preying on chytrid fungus zoospores in the water column. *Daphnia* are also preyed upon by the introduced eastern mosquitofish, *Gambusia holbrooki*, such that where these invasive fish occur in Australia, *Daphnia* are often absent. As such, the invasion of the eastern mosquitofish may have indirectly increased chytrid fungus infection rates in amphibians by eliminating *Daphnia*. Our research investigated this possibility in the presence and absence of vegetation.

We conducted this research in 2L microcosms inoculated with chytrid fungus containing either the eastern mosquitofish, *Daphnia carinata*, both species, and neither species in the presence and absence of vegetation. These experiments informed us whether *Daphnia* is an effective biological control of chytrid fungus. Likewise, whether aquatic vegetation can provide refuges for *Daphnia* in the presence of the eastern mosquitofish. By examining the impacts a species interaction has on chytrid fungus, our research can improve on-ground management methods being employed to mitigate this pathogen.

**What determines choice of retreat site in temperate viviparous geckos: microhabitat structure or thermal profile?**

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Choice of appropriate retreat site has major implications for the survival of ectotherms in fluctuating thermal environments. The chosen retreat site determines how optimally an ectotherm will function in its environment and can be influenced by both abiotic cues (such as thermal cues and/or physical dimensions of the habitat structure) and the biotic component (such as predators). In this study, we investigated whether the retreat site selected by the gecko, *Woodworthia* "Otago/Southland" in southern New Zealand is best explained by microhabitat structure, thermal profile or a combination of both. We measured physical dimensions of rocks occupied by the geckos as well as distance to nearest vegetation. Thermal profile was measured at capture using an infrared thermal camera, as well as by thermocron iButtons that were attached to the rocks over a full year. Our results indicate that both microhabitat structure and thermal conditions influenced retreat sites selected by the geckos. Also, the retreat sites selected varied by season and by life-history stage. We predict that climate warming will eventually force geckos to abandon their superficial summertime retreat sites and move into deeper crevices. However, what the future holds for the geckos if there are not enough deep crevices or alternative microhabitat is a concern.

### Dorsal pattern polymorphism in female Brown Anoles: testing the “male mimicry hypothesis”

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To understand the evolution of polymorphisms in females, many scientists have been interested in the “male mimicry hypothesis”. The evolutionary explanation for male mimicry by females often involves a reduction in sexual harassment, at the cost of higher testosterone levels and lower reproductive success in “male-like” females. Three primary dorsal patterns have been described in female brown anoles (*Anolis sagrei*), but some populations also include a “male-like” pattern. A recent study on female polymorphism in *A. sagrei* proposed that the presence of the male-like dorsal pattern could be maintained as a consequence of relaxed sexual harassment. We tested this hypothesis using two sets of analyses. First, we aimed to determine if female dorsal pattern was truly similar to male dorsal pattern using two different methods: 1) unbiased human vision with no or low previous knowledge of the existing classification, and 2) computer classification of dorsal patterns. Second, we then related the female dorsal patterns to body condition, dewlap size and coloration to support or discount the idea of “male-like” females being viewed as males in the wild. We also related female dorsal patterns to egg production as a metric of reproductive success. In accord with the “male-mimicry hypothesis” we predict male-like females to have larger and more vibrant dewlaps and lower fecundity than the other female morphs.



## Molecular tools help clarifying the conservation value of some anurans from western mediterranean islands

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Amphibians are facing global decline worldwide, as a results of human-mediated disturbance like habitat loss and fragmentation, pollution, spread of pathogens, and release of alien species. At the same time, amphibians are often moved outside their natural range by humans, due to pet trade, for food consumption, or scientific purposes. Sometimes, accidental releases also occur as a result of commercial activities like aquaculture or game fishing. Islands represent biodiversity hotspots for these animals, most of which are endemic species resulting from the long-lasting isolation of populations from the mainland. With the primary aim to detect evolutionary significant units (ESUs) of conservation concern, we genetically characterized insular populations of several Anurans such as the Italian Pool frog (*Pelophylax bergeri*), the Common toad (*Bufo bufo*), the Green toad (*Bufo viridis*), and the Tyrrhenian painted frog (*Discoglossus sardus*) in the Western Mediterranean (Sardinia, Corsica, and the Tuscan Archipelago) by adopting a multi-locus approach involving both mitochondrial and nuclear markers. We detected both introduced and native populations, the latter sometimes showing higher genetic variation than expected and non-obvious patterns of diversification than previously thought. Our results claim for urgent conservation actions to protect such an important cryptic genetic variation found on islands that could act as a biodiversity reservoir even for the threatened mainland populations of the same species.

**Living La Vida T-LoCoH: Using the T-LoCoH method to predict fauna responses to habitat change**

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Understanding the mechanistic processes underpinning the responses of animals to habitat change and restoration is vital to their conservation. Studies of animal responses to habitat restoration typically assess species presence or absence; however, such studies do not account for behavioural or physiological ecology and therefore may not adequately show whether habitats undergoing restoration are effectively facilitating the return of fully functional and self-sustaining fauna populations. We present a case study using VHF/GPS tracking of a sub-adult perentie (*Varanus giganteus*), to demonstrate the effectiveness of the T-LoCoH method of home range construction as a tool in analysing behavioural responses of fauna to habitat change and restoration. Unlike presence/absence studies, provide single point locations of an animal, and the MCP method which provides an invariant estimate of habitat use across the whole home range, T-LoCoH can aid in understanding the complex mechanisms driving the responses of fauna to habitat change, and can show differential use in reference and restoration vegetation through the identification of core-usage areas of the habitat, and areas used infrequently or avoided. T-LoCoH provides an effective tool for assessing habitat specific requirements of organisms and can provide an insight into ecophysiology and behavioural ecology of animals within landscapes undergoing restoration or alteration. The depth of data generated indicates strongly that an understanding of the complex interactions between animals, and their behavioural responses to their environment, is fundamental to their conservation in the face of ever-increasing rates of human induced habitat change and degradation.

**Physiological and molecular aspects regulating the follicular development in the viviparous lizard *Sceloporus torquatus*****Cruz-Cano N<sup>1</sup>**, Álvarez- Rodríguez C<sup>1</sup>, Sánchez-Rivera U<sup>1</sup>, Dávila-Govantes R<sup>1</sup>, Martínez-Torres M<sup>1</sup><sup>1</sup>*Biology of Reproduction, FES- Iztacala, National Autonomous University of Mexico*

Follicular development (FD) is a phenomenon that occurs in the females of all vertebrates it implies an association between follicular cells and the oocyte. In reptiles, as in other vertebrates, environmental cues induce the gonadal activity, promoting estradiol (E<sub>2</sub>) synthesis and vitellogenesis. However, the knowledge of the intraovarian mechanisms orchestrating this event is scarce. The aim of this study was to document the changes in the pattern of E<sub>2</sub> concentrations, estrogen receptor  $\alpha$  (ER $\alpha$ ), and inhibin during the different stages of the FD of *Sceloporus torquatus*. Adult females (n=84) were collected monthly (July 2017-February 2019) in the State Park Sierra de Guadalupe, Mexico. After capture, blood samples of 100  $\mu$ l were collected to quantify E<sub>2</sub> by ELISA assay. Celiotomies were performed to establish the status of FD. A positive relationship between the follicular diameters and E<sub>2</sub> concentrations was detected ( $r=0.88$ ,  $p<0.05$ ). Hormonal concentrations of each stage were compared (ANOVA), significant ( $p<0.05$ ) differences among all the FD stages were found. Lowest E<sub>2</sub> concentrations ( $125.02\pm 79.81$  pg/ml) occurred during the post-breeding season when follicles are  $<2$ mm. With the onset of vitellogenesis, E<sub>2</sub> levels begin to rise until they get at the peak values ( $650\pm 201.4$  pg/ml) and the follicles reach preovulatory diameters of  $8.12\pm 0.59$  mm. After ovulation, a decrease is observed ( $349.54\pm 74.49$  pg/ml). ER $\alpha$  and inhibin expression patterns showed cellular-type specific variation along the FD that remained high during the early stages and declined during the late vitellogenic ones. These results show that the intraovarian mechanisms that regulate FD are conserved among vertebrates.

**Morphological variation of Gliding geckos and other closely related forms**Hernández Morales C<sup>1,5</sup>, Heinicke M<sup>2</sup>, Gamble T<sup>3</sup>, Siler C<sup>4</sup>, **Daza J<sup>1</sup>**<sup>1</sup>Sam Houston State University, <sup>2</sup>University of Michigan Dearborn, <sup>3</sup>Marquette University, <sup>4</sup>University of Oklahoma, <sup>5</sup>University of Texas at Arlington

Gliding geckos from the genus *Ptychozoon* are nested within a clade of chiefly Indo-Pacific gekkotans including *Lepidodactylus*, *Pseudogekko*, *Luperosaurus*, and *Gekko*. Recent molecular analyses indicate the non-monophyly of three of these genera (*Gekko*, *Lepidodactylus*, and *Luperosaurus*), suggesting the need for significant taxonomic rearrangement in this part of the gekkotan tree. Here we study a sample of 31 species representing all major subgroups within this clade. Whole body microCT data (CT and diceCT) was studied using a phenotypic data set of 863 traits. Using the current molecular hypothesis and taxonomy as a framework, we mapped morphological variation using the ACCTRAN optimization. The monophyly of this clade is supported by 3 non-ambiguous traits, 1) frontal interorbital/frontoparietal suture width ranging from 36-40%, 2) frontal supraorbital shelf demarcated medially by narrow shallow longitudinal furrow often bearing row of foramina, 3) frontal supraorbital shelf oriented dorsolaterally. The genus *Ptychozoon* is diagnosed by the lack of contact between the parietal and the supraoccipital, and several other characters may support recognition of this genus (low neural spines, expanded carpus, and extensive body flaps). The node ((((*G. badenni*, *G. grossmanni*) *G. preticolus*) (*Luperosaurus iskandari*, *Gekko vitattus*)) *Ptychozoon*) was supported by having the first sacral rib overlapping the acetabulum. This preliminary study has the potential to provide diagnostic characters required to resolve the taxonomy of this clade of Indo-Pacific gekkotans, and to understand better the morphological changes in gliding geckos.

## **Taxonomic and functional comparison of anuran communities in a megadiverse lowland rainforest in Malaysian Borneo**

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We studied anuran communities in Gunung Mulu National Park, Malaysian Borneo, a site of exceptionally high species richness, with 103 species of frogs recorded so far. We compared species communities in three types of tropical lowland rainforest (alluvial forest, limestone forest, and tropical heath forest/kerangas) with regard to their species richness, species abundances, and species composition. Even though the three forest types are found in close proximity to each other, they differed markedly with regard to species numbers, relative abundances of species, and especially their species compositions. To explain the differences between the communities, we did several tests. First, we measured environmental variables in the sites and tested if differences between species communities are caused by differences in environmental conditions between the forest types. Second, we measured functional traits that describe the ecology, morphology and life history of adults and tadpoles for all anuran species. We then tested if the differences in species diversity and composition between the anuran assemblages are mirrored by differences in functional diversity and composition. We will present the results of the analyses at the conference.

## Diet and Reproductive Biology of Angolan Snakes

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Although the diets and reproductive cycles of many colubroid snakes of southern Africa are well-studied, such data are less complete for Central and West African taxa. This is especially so for the snakes of Angola, where nearly 40 years of civil war has impeded herpetological research. In this study, we examined specimens of 22 species, representing four families of snakes, derived from recent collections from western and central parts of Angola made during both the warm summer months and cooler winter months. For widespread species (e.g., *Bitis arietans*, *Crotaphopeltis hotamboiea*, *Dasypeltis scabra*, *Psammophis spp.*), results are compared with previous studies of these taxa in other portions of their range. The mass, number and identity (to order for arthropods and genus for vertebrates) of prey items are reported. Reproductive data include clutch/litter size as well as male and female gonadal condition. The diverse climate of Angola, combined with its topographic relief (Angolan Plateau versus lowlands) account for much of the variation in reproductive patterns, whereas dietary patterns largely follow those of related taxa elsewhere in sub-Saharan Africa.

### How environmental temperatures affect anurans thermal biology in a tropical forest environment?

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Thermal ecology studies how ecosystems and their organisms can be influenced by environmental temperature. Ectotherms, such as amphibians, are particularly sensitive to thermal fluctuations, since their body temperature depends on the environmental temperature they are subjected to. In order to perform some physiological functions satisfactorily, their body temperature must be within an optimal range in which their physiological performance is enhanced. Whereas in the last seven decades much attention has been given to reptiles' thermal ecology, comparatively, very few studies have focused on amphibians. We sought to investigate the thermal physiology aspects of anurans from mountainous regions of Atlantic Forest, seeking to understand environmental temperature's influence on anuran's body temperature. In these forested regions, large temperature fluctuations occur daily and anurans constitute a very sensitive group to broader temperature shifts. We first discussed how environmental temperatures and body mass can influence the body temperature of the studied species. We found that environmental temperature (here air and substrate temperature) was the primary factor influencing the species' body temperature, whereas body mass also influenced, but to a lesser extent. Subsequently, we evaluated the thermal physiology of three species of the genus *Ischnocnema*, highlighting not only their body temperatures, but also their preferred temperature, minimum and maximal voluntarily tolerated temperatures and a thermal performance curve. We verified that the three studied anuran species have distinct thermal physiologies and do not thermoregulate. Additionally, the local environment temperature seemed to constitute a key factor, limiting to a certain degree the distribution of these species.

**'Green frog in the water' - A herpetological approach on the magico-religious use of the frog in Mesopotamia**

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Inhabiting the rivers and marshes of Mesopotamia the frog was a commonly known part of the fauna between Euphrates and Tigris. Figurative amulets and parts of the animal were used to heal and the frog itself served as a substitute animal. In a variety of early societies frogs were seen as symbols of fertility, rebirth and healing/transformation due to metamorphosis and their ability to live in water and on land. Additionally, they are closely linked to water or rain and therefore associated with cleansing. This paper aims to cross-reference the textual evidence, recently collected and commented by A. Bácskay (Bácskay 2018) with herpetological insights into the medical use of frogs and toads. By the descriptions given in Bácskay 2018, we identified two anuran species occurring in the mesopotamian marshlands, that could have been used as medicinal treatment and substitute animal. We will provide a short summary on their habitat and give insights into their ecology and possible medical value. Together with the presentation of figurative frog amulets from Mesopotamia dating into the 3rd-1st mill. BC including their materials and archaeological contexts, the authors hope to give a comprehensive overview of the magico-religious perception of the frog in Mesopotamia.



## **Behavioral ecology of a common frog (*Rana temporaria*)**

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Successful reproduction is crucial for the maintenance of local populations and species. Therefore, we investigated the mating behavior of the European Common Frog which is a wide spread generalist species. Despite their phenotypical and ecological plasticity, this species decreases in population sizes or even loses local populations. Even though there was a lot of research done on that species, we still lack information about their mating behavior: Questions we asked included e.g. how couples do form and if they do, are they size assorted? Does size assortment have evolutionary benefits? Are males and/or females are choosing their partners to mate with? Are there more successful couples or mothers concerning number and phenotype of offspring?

With different methodological approaches, we could show that size assortment is a common phenomenon in this species and that mostly physiological reasons shape this size assortment (time of migration in differently sized individuals). We could not find an evolutionary advantage in size assortment so far. The number of fertilized eggs is not increasing and multiple paternity is quite common. During mate choice experiments, we found that females are not the passive sex during the explosive breeding period as formerly believed but are able to escape if they are not receptive or possibly dislike the mating partner. That is, they use different strategies to escape, as turning their bodies out of amplexus, emitting release calls or feigning death. In addition, some mothers are more successful in producing offspring and multiple paternity is increasing variability in developmental time.

### Habitat Characteristics and Distribution of Anura Inhabiting in Rice Paddy Fields According to Farming Practices

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Paddy wetlands are a critical habitat for amphibians for breeding, foraging, and resting. Modern rice paddy fields are affected by the application of chemical pesticides and herbicides and, consequently, these changes can influence the distribution of many species. The objectives of this study were to evaluate the habitat characteristics of Anura inhabiting in paddy wetlands and identify major environmental variables affecting their distributions. The study was conducted in the Deaho reclaimed area, where eco-friendly paddy fields and conventional paddy fields coexisted, from Mar to Oct 2018. This study found three Anura species (*Pelophylax chosenicus*, *P. nigromaculatus*, and *Hyla japonica*) and 1,662 individuals. The species abundance was correlated with temperature and humidity positively. Moreover, water-depth and water temperature were significantly different between species. It was also found that they all preferred rice transplanted paddy fields and species used different micro-habitats depending on season. Environmental factors associated with the distribution of Anura were different between the breeding season and non-breeding season. The mean number of *P. nigromaculatus* and *H. japonica* were not influenced by farming practices, but there were more *P. chosenicus* in the environment-friendly paddy fields. The results of GLMM analysis revealed that all three species were affected by air temperature, humidity, water-depth, and micro-habitat characteristics. It is believed that it will be possible to identify why these species responded to different farming practices differently when their relationship with predators, their preferred food resources, and the environment of ditch and levee between rice-fields are thoroughly understood.

## Habitat Environment and Community Characteristics of Anura Inhabiting Rice Paddy Fields in the South Korea

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The endangered herptiles are most densely populated in the paddy wetlands in the west coastal region of South Korea. However, this region is vulnerable to development due to geographical location. This study evaluated the climate, water quality, and physical environments of 40 areas from Mar to Oct in 2016 and 2017 to understand the community characteristics of Anura. Moreover, this study examined the landscape characteristics of the forest rice-fields and those of the flatland rice-fields. Nine Anura species were found in the study area. Temperature, moisture, water temperature, and water-depth were positively correlated with the population size, while air volume and pH were negatively correlated with it. Moreover, NaCl, EC, and TDS were negatively correlated with altitude and the distance from the coast. *Hyla suweonensis* and *Pelophylax chosonicus* preferred flatland rice-fields, and all other species preferred forest rice-fields. The results of self-organizing map(SOM) and random forests analysis revealed that *P. nigromaculatus*, *Lithobates catesbeianus*, *Glandirana rugosa*, and *H. japonica* formed a cluster and they were mainly influenced by forest size and water-depth. *Bufo gargarizans*, *Rana coreana*, and *R. dybowskii* were mainly affected by water temperature and air temperature, while *P. chosonicus* and *H. suweonensis* were mainly influenced by the sizes of forest and farmland. In general, species diversity and richness were high in forest rice-fields, but the two species inhabiting mainly in flatland rice-fields were identified as endangered species. The results implied that the wetland habitat such as flatland rice-fields should be conserved and protected from development.

**Assessment of swab suitability for isolation and metagenomic analyses of *Leiopelma pakeka* skin microbiota**

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Frog skin is a rich substrate for growth of diverse microorganisms including the lethal *Batrachochytrium dendrobatidis*. The contribution of cutaneous microbe communities in mitigating chytridiomycosis has been a breakthrough in saving frogs from this potentially lethal fungal infection. One of the challenges however, in studying frog skin microbiota is keeping the microorganisms viable for subsequent microbial culture and DNA extraction, particularly when samples are transported from remote field locations to the laboratory. In this study, we investigated the efficiency of two different swab systems for collecting skin microbiota. Skin swabs were collected from six individuals of *Leiopelma pakeka* using each swab system. Medical dry swabs (Medwire) have been widely used to detect the presence of *B. dendrobatidis* DNA from frog skin however, may not be ideal for the cultivation of microorganisms. The Copan Eswab Transport System includes a storage tube with Amies Transport Medium that maintains the viability of aerobic microbes. To simulate field collection conditions, the swabs were initially kept at room temperature for five hours and then transferred to different storage temperatures (4°C, -20°C and -80°C for at least 24 hours) to assess the effects of temperature on preserving diverse microbes and their DNA. Numerous bacteria were cultivated and DNA were extracted from both swab systems stored at 4°C, however, fungi were only isolated using Copan Eswab Transport System.

### Brain Size Increase in Pitvipers and its Evolutionary Trade-offs

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In encephalized animals the brain size increase represents more complex behavioral and cognitive patterns, but also impose relevant energetic constraints that are compensated by anatomical changes in other vital systems. We evaluate this evolutionary paradox in pitvipers (Crotalinae: Viperidae) in an interspecific context, and intraspecifically in the species *Bothrops asper* (Crotalinae: Viperidae) to obtain a more accurate interpretational view about brain and anatomical evolution of these snakes. We apply allometric and correlational techniques using the body weight, brain weight, and weight of different organs and tissues from fresh samples collected in Panama, Central America, and we identify two important aspects. First, the brain size increase of pitvipers is strongly constrained by increase of body size. Second, despite the allometric constraint on brain size, the whole body of pitvipers has adaptations to compensate the brain size increase, mainly in the musculoskeletal tissue and the tracheal tissue. Furthermore, the size of the reproductive organs and the visceral fat also compensate the brain size increase in *B. asper*. This suggests that in the pitvipers the selection on body size is stronger than selection on brain size, but the evolutionary process on body size still depend of the constrained brain size. The data for *B. asper* suggests an interplay between reproductive and neurobiological systems where the brain tissue might be compensating the reproductive effort in this species. Thus, although constrained, brain size is fundamental in the anatomical, reproductive, and body size evolution of pitvipers.

## Variation in femur morphology among Gekkotans

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The typical sprawling position of geckos assists them by positioning the body close to the surface, which can be useful while climbing vertical surfaces such as trees or rocks. This type of gait predicts the femoral head to be perpendicular to the main shaft and to be laterally inserted into the pelvis facing the acetabulum. Other geckos have adopted semi-sprawling and nearly parasagittal gaits (e.g., cursorial forms living in hot, arid environments may require that the body to be held away from the substrate). We quantified the variation of femur morphology among 62 species of geckos (six families of limbed gekkotans). Four anatomical landmarks in three dimensions were placed on the right femur of adult specimens: head of femur, trochanter, lateral condyle, and medial condyle. Morphological variation was large in the sample and in the principal component analysis. The first three principal components explained 84.4% of the total variation (53.6%, 20.8%, and 9.8%, respectively). The analysis produced an ordering that allowed us to organize the variation of the femora. Femora changed mainly along PC1, varying from being stout to slender. Diplodactylids have stout bones, whereas some Gekkonids (e.g., *Rhoptropus afer*) showed very slim femora. On the same axis, the head of the femur transforms from being large (more than twice the size of the trochanter) to being small (or subequal in size to the trochanter). This study highlights the anatomical variations on femur morphology, but it is unclear whether correlation is associated through phylogeny or use of habitat between gekkotans.

### **Determinate growth is ancestral in squamate reptiles**

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Body growth is typically indeterminate among ectothermic vertebrates. Indeed, until recently, this growth pattern was considered to be ubiquitous in ectotherms. Our recent observations of a complete growth plate cartilage (GPC) resorption, a reliable indicator of determinate growth, in many species from two advanced clades of lizards (Iguania and Anguimorpha) clearly reject the ubiquity of indeterminate growth in reptiles and raise the question about the ancestral state of the growth pattern. Using micro-computed tomography ( $\mu$ CT), here we examined GPC of long bones in three basal clades of squamate reptiles, namely in Gekkota, Scincoidea, and Lacertoidea. The complete loss of GPC indicating skeletal growth arrest was observed in the majority of the analyzed species. Using a dataset of 178 species representing all major clades of lizards and tuatara, we traced the evolution of determinate growth on the phylogenetic tree of Lepidosauria. The reconstruction of character states clearly suggests that the determinate growth is ancestral for the entire clade and remains common in the majority of lizard lineages. Indeterminate growth evolved independently several times, e.g., in chameleons and agamas, large species of monitor lizards and large species of teiids. We provide evidence that indeterminate growth is neither ubiquitous nor ancestral in lizards. Importantly, determinate body growth evolution is decoupled from evolution of endothermy in this lineage. In light of these data, it is plausible to hypothesise that the last common ancestor of the amniotes was a determinate grower. If true, the evolutionary origin of determinate growth significantly predated that of endothermal thermoregulation.

## Species groups distributed across elevational gradients reveal convergent and continuous genetic adaptation to high elevations

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Although many cases of genetic adaptations to high elevations have been reported, the processes driving these modifications and the pace of their evolution remain unclear. Many high-elevation adaptations (HEAs) are thought to have arisen in situ as populations rose with growing mountains. In contrast, most high-elevation lineages of the Qinghai-Tibetan Plateau appear to have colonized from low-elevation areas. These lineages provide an opportunity for studying recent HEAs and comparing them with ancestral low-elevation alternatives. Herein, we compare four frogs (three species of *Nanorana* and a close lowland relative) and four lizards (*Phrynocephalus*) that inhabit a range of elevations on or along the slopes of the Qinghai-Tibetan Plateau. The sequential cladogenesis of these species across an elevational gradient allows us to examine the gradual accumulation of HEA at increasing elevations. Many adaptations to high elevations appear to arise gradually and evolve continuously with increasing elevational distributions. Numerous related functions, especially DNA repair and energy metabolism pathways, exhibit rapid change and continuous positive selection with increasing elevations. Although the two studied genera are distantly related, they exhibit numerous convergent evolutionary changes, especially at the functional level. This functional convergence appears to be more extensive than convergence at the individual gene level, although we found 32 homologous genes undergoing positive selection for change in both high-elevation groups. We argue that species groups distributed along a broad elevational gradient provide a more powerful system for testing adaptations to high-elevation environments compared with studies that compare only pairs of high-elevation versus low-elevation species.



**Molecular phylogeny and biogeography of the Japanese toads (*Genus Bufo*)****Fukutani K<sup>1</sup>**, Matsui M<sup>1</sup>, Nishikawa K<sup>1</sup><sup>1</sup>*Kyoto University*

Using mitochondrial Cytochrome b sequence, we investigated phylogenetic relationships and estimated the historical demography of the toads endemic to mainland Japan. The toads consist of two species, *Bufo japonicus* breeding in the still water and *B. torrenticola* breeding in the stream. Further, *B. japonicus* has two subspecies, *B. j. japonicus* distributed in western Japan and *B. j. formosus* distributed in eastern Japan. The two subspecies are distributed parapatrically however *B. torrenticola* is distributed sympatrically with *B. j. formosus* in some areas. Our present result showed that the Japanese toads from mainland formed a major clade, which was divided into two clades, eastern and western clades. Each of these two clades had three distinct subclades, one of which in the western clade corresponded to *B. torrenticola*. Based on genetic distances observed among subclades, including *B. torrenticola*, *B. japonicus* was suggested to be divided into several species. However, in order to revise the taxonomy of the Japanese toads, we need to examine the possible gene flow among these subclades. Results of divergence time estimation and population genetic analysis indicated that multiple factors contributed to form diversification of the Japanese toads. The pattern of genetic differentiation in the Japanese toads has been affected by geohistorical events and paleoclimate changes associated with the formation of the Japanese archipelago.

**A new species of the genus *Larutia* (Squamata: Scincidae) from Gunung Penrissen, Sarawak, Borneo**

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Skinks of the genus *Larutia* Böhme 1981 are fossorial or semifossorial and have no limbs or tiny limbs. The *Larutia* encompasses eight species from Malay Peninsula, Sumatra and Borneo. Only one species of the genus, *L. puehensis* is known from Borneo. The species was described based on only the holotype, which is the sole specimen collected from Borneo so far. In March 2018, we collected a specimen of *Larutia* species from Gunung Penrissen (Mt. Penrissen), Kuching Division, Sarawak, Malaysian Borneo. The specimen differs from all other known congeners by the morphological characters including small adult body size (SVL of adult male: 84mm); 22 longitudinal scale rows around midbody; the first pair of chinshields contacting second infralabial and the second pair of chinshields separated from infralabials by an elongated scale; two subdigital lamellae on second toe; body without yellow or pale bands or spots. Molecular analysis on the mitochondrial ND1 gene indicated that the specimen belongs to the sister species of *L. seribuatensis*, distributed in Tioman Island. It will be the ninth species in the genus and the second species of *Larutia* in Borneo.

## Comparison of venom proteome and venom-gland transcriptome of *Hydrophis curtus* from the South China Sea

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We executed an omics-analysis of the venom of *Hydrophis curtus* from the South China Sea. Proteomics analysis revealed three protein families [three-finger toxins (3-FTx), phospholipase A2 (PLA2) and cysteine-rich secretory protein], and venom-gland transcriptomics analysis revealed 22 protein families from 56 unigenes. 3-FTx (46.89% in proteome/27.15% in transcriptome) and PLA2 (52.19%/59.71%) were identified as the most abundant families in venom proteome and venom-gland transcriptome. A total of 1283 phosphorylated sites and 69 N-glycosylated sites on venom proteins were predicted from 56 unigenes, and strong signals of phosphorylation and N-glycosylation were detected in the venom proteins by western blot. Furthermore, *H. curtus* venom expressed strong lethality (i.p. LD50: 0.16 µg/g) in mice, and showed notable activity in PLA2 but weak activity in L-amino acid oxidase, 5' nucleotidase or SVMP. ELISA analysis revealed that the commercial *Naja atra* antivenom presented a stronger cross-reactivity with *H. curtus* venom proteins than the *Bungarus multicinctus* antivenom. Western blot showed that not all of the venom components could be recognized by *N. atra* and *B. multicinctus* antivenoms; the venom components with low molecular weights expressed lower cross-reactivity with antivenoms than that with higher molecular weights, although they possessed higher abundance. Therefore, design of a species-specific antivenom of *H. curtus* venom with the attention on enhancing the immune response of the components with low molecular weights should be encouraged.

**Somewhere over the rainbow: Cryptic evolution and description of species from Two keeled rainbow skink complex**

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In the last years, Australian herpetologists have been assessing an extensive mtDNA and morphological data of cryptic species in the northern Australia. Methods of delimitation of species are widely used and could guide to separate genetic groups/population that could represent different species, particularly in taxa with conserved morphology. Here we combine an integrative approach to study the evolution of cryptic species in the *Carlia* amax species complex. This complex is characterised by two keeled dorsal scales, differing from other species of the genus, widely distributed at the Australian Monsoonal Tropics, a savanna biome. Previous studies using exon capture data showed at least four species in this complex. Now, adding geographically extensive SNP data and existing mtDNA, morphological and ecological data we evaluate this complex of cryptic species. Even being morphologically similar we found significant morphological differences, especially in the closed relative species. Our SNP data supported the split in four populations with almost no sign of introgression. Our test of niche dissimilarity between sister taxa showed significant differences in their niches, where the most important variable change for each taxa. However, because they still have conserved morphology, we could find any significant scales differentiation between taxa. Here we improve the delimitation and named the cryptic species in this group, solving major taxonomic boundaries that struggled in the previous studies

**Observations of the reproductive behavior of the Guatemalan Spike-thumb Frog (*Plectrohyla guatemalensis*)**

**Gonzalez S<sup>1</sup>, Marmol A<sup>1</sup>**

<sup>1</sup>*Universidad Del Valle De Guatemala*

We conducted a monitoring program for two populations of *Plectrohyla guatemalensis* for three consecutive rainy seasons in two different sites at Sacatepéquez, Guatemala. Part of our observations on the frog populations we found included noteworthy reproductive behavior. Our findings suggest that the species' reproduction is closely linked to the crevices of rocks in streams, inside which the males were found vocalizing to attract one or more females that lay their eggs which are then fertilized and guarded by the male until they hatch. We believe the species to present male-male competition to gain control of these reproduction sites, evidenced by the finding of males with scars consistent with injuries caused by the spikes at the base of the males' thumbs. Also, with the help of local wardens, we managed to record vocalizations as early in the year as mid-January, extending the previously reported reproductive period for this species. We consider that these observations can aid the conservation and restoration of specific habitat for the reproduction of this species. Therefore, we encourage the conservation of rocky streams with adequate forest coverage to be an important action to protect this species' reproductive populations within their distribution.

**Phylogeny of the amphisbaenia (reptilia: squamata) based on anchored phylogenomics and morphological dataset**

**Graboski R<sup>1</sup>**

<sup>1</sup>*Museu Paraense Emílio Goeldi*

Amphisbaenians are classified as a suborder of Squamata, and comprises of 193 nominal taxa, with distribution in Africa, Americas, Middle East and Mediterranean region. Traditionally they are classified in six families: Amphisbaenidae, Blanidae, Bipedidae, Cadeidae, Trogonophidae and Rhineuridae. Despite the advances in systematics and taxonomy of this group in the last century, no study has used a total evidence approach using genomics and morphological dataset. Here we provide the first comprehensive reassessment of the phylogeny of Amphisbaenia and their phylogenetic position within Squamata. We explore two distinct methods for phylogenetic inference (concatenation and species tree) using Anchored Phylogenomics (390 loci) sequencing methods. We analyzed the most comprehensive taxon sampling (102 species belonging to the amphisbaenians diversity) available until the present. Additionally, we performed a total evidence analysis combining a morphological matrix with Anchored Phylogenomics dataset. Our phylogenetic results show that most families of Amphisbaenia are strongly supported. Most groups within Amphisbaenidae family are retrieved with very low support values.

**Estimating contact rates of Eastern newts (*Notophthalmus viridescens*) at differing temperatures, densities and habitat structure**

Peterson A<sup>1</sup>, Bohanon M<sup>1</sup>, Carter E<sup>1</sup>, Bajo B<sup>1</sup>, Watcharaanantapong P<sup>1</sup>, Miller D<sup>1,2</sup>, Surlles J<sup>3</sup>, **Gray M<sup>1</sup>**  
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The recently discovered pathogen *Batrachochytrium salamandrivorans* (Bsal) is responsible for driving ongoing declines in salamander populations in Europe. While this pathogen has not yet been detected in North America, the introduction of Bsal into novel regions is likely. Transmission of Bsal can occur via direct contact, thus a key component in understanding the ecology of this pathogen is to identify how contact rates in host species vary across realistic conditions. Such work can inform management strategies that may help limit Bsal spread when introduced into novel populations. In this study, we placed adult Eastern newts (*Notophthalmus viridescens*), a widespread North American salamander species known to be susceptible to the Bsal pathogen, into 1-m<sup>2</sup> aquatic trough enclosures at varying densities (2, 4, 8 and 16 animals/m<sup>2</sup>). We also experimentally manipulated habitat structure (0, 4, 8, 16 plants/m<sup>2</sup>) and water depth (3", 6", 9", 12") using a Latin-Square design. We utilized video recording of animals from eight diel periods to estimate how daily contact rates vary across these conditions at three biologically relevant temperatures (6°C, 14°C and 22°C). The results of this experiment can be used to parameterize models of Bsal transmission, and can facilitate identification of potential management strategies (such as altering host density, habitat structure, water depth or temperature) to limit Bsal spread if introduced into North American salamander populations.

**Changes in Eastern Newt Behavior Associated with *Batrachochytrium salamandrivorans* (Bsal) Chytridiomycosis**

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*Batrachochytrium salamandrivorans* (Bsal) is an emerging fungal pathogen linked to population declines of salamanders in Europe. While research concerning Bsal is expanding, little is known about how infection influences behavior. Understanding changes in behavior could lead to early detection of the pathogen in the wild during population monitoring of hosts. There may also be ecological implications if behavioral shifts lead to changes in fitness. To better understand if Bsal influences behavior, we exposed adult eastern newts (*Notophthalmus viridescens*) to one of four Bsal zoospore doses ( $5 \times 10^3$ - $6$ ) and monitored food consumption and movements. After exposure, the newts were held at 14C for 45 days in 300mL of water with a PVC cover object. Each newt was fed bloodworms according to their body mass, and percent consumption was measured. We estimated newt movements by assigning a habitat-use score: newt submerged and under the cover object, submerged but not using the cover object, partially submerged, or newt out of the water. We found that as Bsal chytridiomycosis progressed newts became less likely to be under the cover object, more likely to be out of the water, and they consumed fewer bloodworms. These results suggest that salamander behavior and possibly energetics could be influenced by Bsal infection, which could play a role in the detection of infected hosts and contribute to the epidemiology of Bsal.



**Deep insight into lepidosaurian heart: Tuatara and the others**

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Lepidosaurian hearts stand in the shade of the hearts of homeothermic vertebrates – mammals and birds. However, among lepidosaurian reptiles there should be convergences in the heart anatomy according to life-histories of particular species. Therefore, to study the association among the structures of reptilian heart (sinus venosus, atria, and ventricle), we focused on lepidosaurian models, where the heart structures are composed differently. Chosen lepidosaurian species were investigated across the phylogenetic tree – *Sphenodon*, as the sole survivor of the order Rhynchocephalia and the closest relative of squamate reptiles, and several squamate species (*Bitis*, *Boa*, *Chondrodactylus*, *Gekko*, *Iguana*, *Pogona*, *Tupinambis*, and *Varanus*). We used histological methods (e.g. Trichrome or Picro Sirius Red - PSR) and immunohistochemistry (e.g. Human Natural Killer-1 and different myocardial markers) to uncover the inner structures of adult lepidosaurian hearts. Tested species were also analyzed by  $\mu$ CT and confocal microscopy for better detection of chosen markers in ventricular septa. In *Sphenodon* PSR stained collagen deep in the spongy ventricle without any sight of ventricular septa.  $\mu$ CT showed us distinctions in the structures of sinus venosus, atria, and ventricle among lepidosaurian species according to their life-histories. For example, athletic animals, such as *Varanus* or *Tupinambis*, showed visible analogy of the three ventricular septa (muscular ridge, bulbuslamelle, and ventricular septum). These differences among lepidosaurians are possibly linked to life-histories but they can also reflect position of particular species in phylogenetic tree. In the absence of fossil evidence, this is the closest approach to understanding the evolution of the heart.

## **A global directory of over 40,000 dissertations and theses on reptiles and amphibians**

**Grieneisen M<sup>1,2</sup>**, Hassapakis C<sup>2</sup>, Clark H<sup>3</sup>

<sup>1</sup>*University Of California*, <sup>2</sup>*Amphibian & Reptile Conservation, co-editor*, <sup>3</sup>*Colibri Environmental Consulting*

Dissertations and theses are the products of a substantial investment of resources in scientific research. Collectively, theses contain a wealth of research data, much of which does not appear in peer-reviewed publications until years after the thesis is available, if ever. The lack of formal publication is often due to the time constraints on thesis authors (as recent graduates) rather than inferior data quality. The "electronic thesis and dissertation" (ETD) movement has prompted 1000s of universities worldwide to establish open-access thesis repositories, which greatly improve the accessibility of theses.

For this project, information on over 43,000 theses (about half from institutions in the USA, and half from non-US institutions) has been extracted from numerous university library catalogues and national thesis repositories (e.g., Canada, India, Kenya). This compilation documents the contributions of thousands of herpetologists in pursuit of higher degrees; and it includes theses that are of strictly herpetological interest, in addition to those where herpetofauna are used as model organisms for studies on anatomy, physiology or molecular biology.

Currently, certain geographic regions are poorly represented, due to limitations in either our linguistic skills or the geographic coverage of electronic databases. We are actively recruiting collaborators to help us improve the coverage in those areas, particularly in Asia, Latin America, and the Middle East.

The presentation will demonstrate the extent of herpetological data that are only available in theses, and show the geographic and topical diversity of this unique bibliographic collection.

**Geographic features and Pleistocene glaciations affected a southern Argentinean lizard species group (genus *Liolaemus*)**

**Grummer J**

Our knowledge of phylogeographic patterns is skewed towards northern hemisphere taxa, with a knowledge gap in relation to Pleistocene effects on Southern Hemisphere taxa. We collected SNP data for 169 individuals in the *Liolaemus fitzingerii* species group of lizards in southern-central Argentina to test the hypothesis that the last glacial maximum affected geographic distributions and therefore evolutionary histories of these species. We did this by identifying population-level boundaries, estimating geographic patterns of migration and genetic diversity, and inferring demographic histories. Although current taxonomy recognizes twelve species in this group, our analyses identified six genetic clusters. Phylogenetic relationships between populations are weakly supported – only a single clade of the southern-most two populations, corresponding to *L. camarones*, *L. fitzingerii* and *L. xanthoviridis*, received strong support. Our results indicate that *L. camarones* and *L. dumerili* are not valid species, and are uncommon in that more species are recognized in this group with morphological than genetic data. Migration analyses identified large geographic features such as Somuncura and Canquel plateaus along with the Chubut River as barriers to gene flow. Genetic diversity was generally lower in individuals inhabiting western (higher elevation) and southern (higher latitude) regions. Demographic model testing in four parapatrically distributed population-pairs identified gene flow between all populations and recent population bottlenecks (<60,000 years before present) in the four most southerly distributed populations. Taken together, our results support the hypothesis that populations retreated and expanded due to recent glacial cycles, and add to the collective understanding of phylogeographic patterns of temperate Gondwanan taxa.

## Using Geometric Morphometrics to Study Lizard Adhesive Toe Pad Shape Across Macro and Microevolutionary Scales

Hagey T<sup>1</sup>

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Adhesive toe pads arose at least three times in lizards (geckos, anoles, and skinks) and have been the subject of intensive biomechanics and adaptive functional morphology research. Much of our understanding of adhesive mechanics come from the Tokay gecko (*Gekko gecko*) while much of our ecological understanding of how adhesive pads help species thrive come from Caribbean anoles. Given the extensive morphological variation in toe pads across geckos, anoles, and skinks, much of our evolutionary understanding has been hampered by the complexity and variation the adhesive system. Here I will describe approaches we've used to study morphological variation in pad shape at three different scales, macro-evolutionary, meso-evolutionary, and micro-evolutionary. We quantified toe pad shape across the Gekkota infraorder to investigate macroevolutionary convergent evolution, toe pad shape within the Hemidactylus genus highlighting how a single origin of toe pads likely changed and adapted over time, and infraspecific toe pad shape in *Anolis cristatellus* highlighting adaptive change related to the colonization of urban microhabitats.

**Increased predation threatening endangered Hungarian meadow vipers (*Vipera ursinii rakosiensis*)**

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The Hungarian meadow viper (*Vipera ursinii rakosiensis*) conservation program started to reintroduce captive bred vipers in 2010. Over the past 8 years altogether 504 vipers were released to six habitats in Kiskunság and Fertő-Hanság National Parks in Hungary. During regular monitoring, we observed 15% of the released vipers at least once again, including 26 gravid females and 48 additional newly registered juveniles or subadults. The longest period between release and last observation was 1575 days.

In order to apply a remote tracking method, pre-programmed radio-tags with a detection range of 100-200m were surgically implanted into the abdomen of selected vipers. Generally, five snakes were tagged within each released group, resulting 35 tagged snakes. Each snake was localized at least once a week. Snakes had an average Minimum Convex Polygon of 0.43 ha (max. 1.51 ha), with an average distance between locations of 324 m (longest 921 m). Only eight snakes (23%) managed to overwinter. In 21 cases (60%) the snakes were killed by predation, half of them by avian predators like Common buzzards (*Buteo buteo*) or Harriers (*Circus sp.*) and the other half by mammals, most likely Red fox (*Vulpes vulpes*) and Badger (*Meles meles*). The most sensible periods to predation were the week after release and the early spring and late autumn periods. We suspect that systematic immunization against rabies and success of raptor conservation significantly increased predation on reptiles species over the last decades in Hungary, threatening long-term perspectives of Hungarian meadow viper populations.

With the ambition to significantly improve the conservation status of the species and its habitats, a new LIFE-project is launched in 2019, targeting such problems like increased predation.

## Salinity aversion of temperate zone amphibians: implications for anthropogenic salinization

### Hazard L<sup>1</sup>

<sup>1</sup>*Montclair State University*

Global climate change may lead to increased salinization of freshwater habitats through local changes in winter snowfall (and therefore application of road de-icers), as well as increased seawater incursions into freshwater habitats. Salts may contaminate local watersheds to levels high enough to potentially impact amphibian populations, especially during early spring when winter de-icers enter aquatic systems. We tested adults of several amphibian species from the northeastern United States to determine whether they showed behavioral aversion to increased salinity, and if so, at what threshold concentration. Species included fully terrestrial species, streamside species, obligate vernal pool breeders, and habitat generalists. Animals were evaluated using binary salinity choice trials and salinity gradient trials of 10- or 60-minute duration. Some species showed immediate aversion to even low concentrations while others took longer to show a response. In general, across species aversion was shown in response to hyperosmotic solutions but not hypoosmotic solutions, suggesting that risk of water loss may drive salinity aversion. However, larval amphibians are often sensitive to lower salinities, and selection of inappropriate breeding and oviposition sites by adults could greatly decrease egg and larval survival. Species with higher behavioral thresholds may attempt to breed in habitats unsuitable for successful egg and larval development. Further research is needed on the responses of breeding adult amphibians to salinity increases under natural conditions. These results will help predict current and future impacts of increased habitat salinity on amphibian communities.

## Human Culture can lead to Exploitation of Amphibians and Reptiles

**Heaton R<sup>1</sup>**

<sup>1</sup>*ZSL Trustee*

This study looks at human cultural representations of herpetofauna in relation to present-day threats to species and their conservation needs.

The earliest human images of herpetofauna are finger paintings of snakes on cave walls. The spectacular "Dome of Serpents" at Rouffignac and the giant fang-headed serpent of Baume Latrone, both in France, are dated at between 20 to 30 thousand years ago. Other images of snakes from early human cultures occur in cave art from Africa and Australia, and in images from Native American culture. Snakes have retained great cultural importance as ritual objects.

The frog-headed Hequet was goddess of childbirth in ancient Egypt, the Aztec toad was earth mother goddess and the Moche people of Peru worshipped the frog. Chinese earthquake detectors have used bronze frog resonators to alert since 300 AD. Today the endangered Panamanian Golden Frog retains strong cultural significance. In fairy tales and puppetry frogs are strong characters. Even stronger frog characters are imagined in one of Monty Python actor Michael Palin's Ripping Yarns adventures, "Across the Andes by Frog"!

In the 1700s, Gilbert White's tortoise Timothy was at the beginning of a pet trade to colder northern climes that saw millions of animals die. The Teenage Mutant Ninja Turtles craze of the 1980s created a trade in live turtles, many of which died, while any survivors created invasive species issues.

Cultural significance does not always end well for herpetofauna species. Are there ways that conservationists today can reverse this trend?

## Temperature and amphibian chytridiomycosis: Review and synthesis

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Chytridiomycosis, a devastating amphibian disease caused by the fungal agent *Batrachochytrium dendrobatidis* (*Bd*), is influenced by a range of environmental conditions, particularly temperature. Since host and pathogen are ectothermic, temperature has strong influence on the biology of both. However, the resulting host-pathogen interaction is not entirely straightforward. In this review, we aimed to evaluate the breadth of temperature-related factors influencing the interaction, while identifying current research gaps, by systematically evaluating the empirical peer-reviewed *Bd* literature since 1998. We show that the role of temperature is complex and has strong influence over a range of factors including immunology, skin microbiota, pathogen biology, and host behaviour. We summarise and analyse mechanisms proposed to be highly influential over the host-pathogen interaction. Behavioural fever, the altered thermoregulatory behaviour of infected hosts, is a potential host strategy to clear infection—however, evidence for its existence is limited and importantly, the behaviour is environmentally constrained for a majority of hosts. The chytrid-thermal-optimum hypothesis, focusing on the thermal niche where *Bd* has the strongest impact on hosts, likely drives infection, though host thermal optimum drives disease risk in optimum direction. Evidence for this interaction is mounting through evidence of thermal preferences of hosts, both at the species and individual level, and the thermal mismatch hypothesis, positing that disease risk is greatest with strong temperature deviations. Understanding the role of temperature and formulating generalisable predictions is vital for mitigating disease risk in future climate scenarios. We discuss implications for management and propose future research directions.



**A course-based undergraduate research experience (CURE) in amphibian ecology: interaction between invasive plant leaf litter and NaCl on two model amphibians**

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Human activities are rapidly changing natural environments, often with harmful consequences for native communities. In particular, the introduction of invasive species and chemical contaminants to wetlands can negatively impact amphibian species, though few studies consider their joint effects. Towards this goal, we tested how leachates from invasive plants and road salt impacted two model amphibians (northern leopard frogs and African clawed frogs). Specifically, we examined the effects of native and invasive leaf litter leachate and sublethal NaCl concentrations on amphibian development, size, and tolerance to a lethal concentration of NaCl. Exposure to invasive leaf litter and sublethal NaCl both accelerated hatching time in leopard frogs, but neither affected hatching time in clawed frogs. Exposure to invasive leaf litter also led to reduced mass and tolerance to lethal NaCl concentrations in leopard frogs, but had no effect on mass and led to increased tolerance to lethal NaCl concentrations in clawed frogs. These findings suggest that invasive leaf litter leachate impacts these amphibian species differently, being more stressful to leopard frogs than clawed frogs. Further, we demonstrate that the presence of a pollutant may augment the effect of invasive leaf litter on amphibians, highlighting the need to consider concurrent stressors in invasive species management. This study was part of a course-based undergraduate research experience (CURE), in which an undergraduate ecology class addressed a research question with unknown outcomes. In this poster, I will also discuss the development, implementation, and outcomes of CUREs focusing on solving environmental problems at Binghamton University, NY, USA.

### Importance of water to abundance and species richness of herpetofauna in the Mexican Mayan jungle

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Herpetofauna occurring in the Mayan jungle is one of the richest assemblages in the Americas. The Calakmul Biosphere Reserve (UNESCO World Heritage Site of Culture and Nature due to the forest of outstanding biodiversity) in Mexico contains 89 species, including some Yucatan endemics. Due to the lack of rivers and permanent waterbodies, species diversity is heavily affected by distance from semi-temporary aquatic sites (formed during the wet season and acting as a crucial resource for a wide variety of species). Visual encounter surveys for herpetofauna were carried out in the mornings and evenings between June and August from 2014 to 2018. Relative abundance (individuals/km) significantly differed between 2014 and 2018 in amphibians and lizards, but not in snakes ( $p = 0.100$ ) and turtles ( $p = 0.680$ ). Species richness (species/km) only significantly differed ( $p < 0.001$ ) in amphibians, lizards and snakes. Abundance and species richness were highest in 2014, with a significant drop thereafter likely as a response to an overall decrease in water availability. Furthermore, *ad libitum* surveying records in waterbodies indicate a decline in overall herpetofauna abundance and diversity of both reptiles and amphibians paired with a significant reduction in water surface. Our results suggest that herpetofauna species in Calakmul are being affected by the ongoing drought, but it is not yet clear if the change in water distribution results in a decline or simply reduced detectability due to alteration in habitat preferences and activity patterns.

**Population genetic structure of *Karsenia koreana*, the only Asian plethodontid**

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Korean crevice salamander (*Karsenia koreana*) is endemic to South Korea and is the only plethodontid salamander species found in Asia. Despite its uniqueness and academic importance, population analysis of this species has not been performed yet. In this study, we collected samples from all known regions throughout South Korea, and the distribution of genetic variability was analyzed using newly developed fourteen microsatellite loci. Considering that the distribution of this species is very restricted and only a small number of individuals are found, the genetic diversity was rather higher than expected. According to our microsatellite results, the populations could be subdivided into two genetic clusters (Pyeongchang + Jeongseon and Jinan + Jeongeup), and the level of genetic difference between the clusters was substantial. Isolation by distance was not found. Because this species does not travel a long distance and live only in watery or humid environments, small landscape structures are assumed to have provided sufficient barriers spatially restricting each cluster. In the future, it is necessary to investigate whether the genetic drift caused by the small population size of this species affected the population structure observed in this study.

**Assortative mating and color polymorphism in Stejneger's grass lizard (*Takydromus stejnegeri*)**

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Color polymorphism is considered relevant to mate preference and plays a critical role in sexual selection. Stejneger's grass lizards (*Takydromus stejnegeri*), endemic to Taiwan, is characterized for their polymorphic coloration which could be categorized into green or brown types in both sexes. This phenomenon attracted our interests to investigate the role of color polymorphism in their mate preference. From 2017 to 2018, we conducted a capture-mark-recapture program and marked 366 males and 336 females. The result suggested that the body colors vary with their breeding seasons. In non-breeding season, the percentage of green type was 11% among all the males and it increased to 20 % in the breeding season. In contrast, green type accounted for 29% of the females in non-breeding season and rose to 57% in the breeding season. We further examined their mate choice to explore whether green color is preferred. The result indicated that green females show preference toward green males. This result partially supports the hypothesis of color-assortative mating. We further inferred that assortative mating might explain the maintenance of color polymorphism in Stejneger's grass lizards.

**Expression analysis of mitofusion 2 mRNA in cold stress of frog. *Rana dybowskii***

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The *Rana dybowskii* distribute in northeast region of China which have seasonally cold climates. During winter they survival freezing by biosynthesizing carbohydrate cryoprotectants such as high concentrations glucose into blood and some tissues.

Our before study have found the *R. dybowskii*'s skin have high express glucose transporter 1(GLUT1) and GLUT4 mRNA in cold hardiness,that imply the tissues can increase glucose uptake in cells when it stimulated by cold stress.this provide a novel insight into the role of GLUTs in cryoprotectant synthesis and cell protection in frog.

Recently, we clone the mRNA of mitofusion 2(mfn2) gene in DH-5a bacteria with pUC18 plasmid. Express the mfn2 protein in saccharomyce with pPIC9K plasmid.Realtime PCR results show the mfn2 expresses dynamic ralated with the GLUTs family protein during low temperature stimulated. Mfn2 can partially affect the expression level of glut4 in skin cells, thereby affecting skin cells to change the intracellular liquid crystal osmotic pressure against low temperature stress by using sugar in plasma and extracellular fluid. This study we firstly indentified and characterized mfn2 in amphibious and found a new significant funtion of it.

## Genetic Diversity and Relationship of Schlegel's Japanese Gecko (*Gekko japonicus*) Across Korea, Japan, and China

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To verify the genetic diversity and relationship among *G. japonicus* populations across Korea, Japan, and China, we collected total 411 geckos from five populations in Korea [Busan (NamS, Comp NatH), Mokpo, Kimhae] and five in Japan [Tsushima, Fukuoka, Innoshima, Kobe, Kyoto, Kobe] and two in China [Yancheng, Wenzhou] and analyzed their mitochondrial DNA sequences (Cytb and ND2) and seven nuclear microsatellite loci. The overall number of haplotypes of Cytb + ND2 was 18, and Chinese populations had greater mean number of haplotypes as 7.6, comparing to 2.8 for Japanese and 2 for Korean populations. Like mtDNA results, mean number of alleles for each locus per population was small as 4.1 (3.8 for Chinese, 3.7 for Japanese, and 2.9 for Korean populations). Populations had relatively small among-population variations in AMOVA tests on microsatellite and there was no significant relationship between genetic ( $F_{ST}$ ) and geographic distances across the three countries. Although we found possible gene flows between some populations, genetic relationships among all investigated populations were not clearly resolved. Considering historical limits, physical barriers, and current low genetic diversity across the three countries, anthropogenic introduction might play an important role for current distributions of *G. japonicus*. Our results also imply that gene flows among the countries might be frequent, and that the secondary dispersal or introduction is also occurring. This study was supported by the National Research Foundation of Korea (NRF, 2016R1D1A1B03931085)

### Habitat Suitability Models of Korean Crevice Salamander (*Karsenia koreana*) at Forested area in South Korea

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We studied the relationship between Korean crevice salamander (*Karsenia koreana*) and environmental factors and contributed to the provision of basic ecological data for future conservation of populations. The purpose of this study was to derive a habitat suitability model for the *K. koreana* using presence-absence data for the species and habitat factor information, and to identify the environmental factors that greatly influence *K. koreana* presence. In 2015 and 2017, 179 survey plots within five forested areas in Daejeon Metropolitan City were surveyed for the presence of *K. koreana* using line transect surveys and time constrained surveys. Three categories of environmental factors were also measured in each habitat. The habitat suitability model was derived from this information, and factors that significantly affected the presence of *K. koreana* were determined. The presence of *K. koreana* was detected at 77 survey plots within the five forested areas. In terms of forest structural factors, the amount of coarse woody debris and the percentage covers of various substrate types were found to be major factors in determining *K. koreana* presence. pH and leaf litter depth were found to be significant soil condition factors, and water temperature, dissolved oxygen, and channel width were determined to be significant water condition factors. These factors will need to be referenced and utilized for the future conservation of the species, and future studies will need to identify further correlations among these factors.

**Behavioral plasticity of microhabitat selection of invasive bullfrog tadpoles reduces predation by local predatory fish**

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Predation risk is among the key forces driving the evolution of escape behaviors and related phenotypes. Successful avoidance of predators results in increased survival rate and fitness. Here, we evaluated the anti-predator functions of microhabitat preference and behavioral plasticity in *Lithobates catesbeianus* tadpoles through manipulative experiments. We placed wild-caught tadpoles into one of three microhabitat treatments together with a local predatory fish (*Channa argus*): non-vegetated, submerged vegetation, or floating vegetation. The survival rate of tadpoles with submerged vegetation was higher than that of the non-vegetated microhabitat. In the habitat preference experiment, “unhurt” and “wounded” wild-caught tadpoles were given a choice. Both types of tadpoles preferentially selected the submerged vegetation compared to the non-vegetated habitat. In contrast, only wounded tadpole significantly preferred floating vegetation over non-vegetated habitats. We conclude that *L. catesbeianus* tadpoles can adjust their anti-predatory behavior according to previous experiences, and thus, demonstrate behavioral plasticity in using microhabitats that they do not use in the absence of predators. This behavioral strategy is likely to directly increase their ability to avoid predation and may be an additional factor to their rapid colonization of habitats worldwide.



## **A critical appraisal of environmental DNA as a tool in aquatic conservation**

**Kielgast J<sup>1</sup>**

*<sup>1</sup>University Of Copenhagen*

The basis of any conservation effort is knowledge on the biology and distribution of a given target organism or community. However, in many cases species of conservation concern are cryptic of nature and occur at low densities. This makes lack of solid distribution and abundance data a pervasive limitation to optimal conservation strategies. In recent years environmental DNA based monitoring has been presented as a solution to address this in aquatic ecosystems and amphibians have been a focal group at the forefront of this development. It has been demonstrated for numerous species that DNA in water samples can be used to document species presence and potential benefits of increased reliability, sensitivity and repeatability has been advocated. The appealing simplicity - water samples instead of experts - has quickly led the way from concept to implementation in practical nature management. I will present an overview of developments in the field and discuss major challenges on the current research agenda. Environmental DNA may likely replace some aspects of conventional amphibian monitoring regimes in the near future but the new possibilities should be embraced with a critical mind-set.

**Using conspecific call to increase the efficiency in capturing the invasive American bullfrog (*Lithobates catesbeianus*)**

**Kim A<sup>1</sup>**, Chuang M<sup>1</sup>, Jang Y<sup>1</sup>

<sup>1</sup>*Division of EcoScience, Ewha Womans University*

Invasion by American bullfrog (*Lithobates catesbeianus*) has become one of the serious ecological issues that threaten global biodiversity and lead to severe economic losses. They can predate native species directly, or compete with habitat and food resource, also modify the behavior of natives indirectly. In Korea, bullfrogs were imported as a food source in 1957 and 1971 and threaten local amphibian populations including endangered *Pelophylax chosonicus* and *Dryophytes suweonensis*. However, there is very few research about controlling of bullfrog population has been done. Developing strategies to eradicate the localized bullfrog populations is needed to minimize the effect of bullfrogs. The goal of this study was to test the efficiency of funnel traps equipped with attractants. In 2018, we weekly monitored the bullfrog population from 48 sites for nine trials in South Korea and recorded 1061 individuals during surveys. The appearance and calling activity were affected by environmental variables, including temperature and the Julian day. From the fourth, fifth, and sixth monitoring trials, we also set funnel traps at the sites with treatments of control, fluorescent lights, and conspecific call. We captured 55 adults with the capture rate of 38.2 individual per hundred trapping night. The captured rate was positively correlated to the local population size and was significantly higher in the treatment with acoustic stimuli. Our findings suggested that set traps with conspecific calls in the core distribution area of bullfrogs could increase the capture. It can be beneficial in controlling the population size of invasive bullfrogs in Korea.

## Genetic and phenotypic variation used to identify populations of endangered green gecko (*Naultinus*)

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Two species of green gecko (*Naultinus stellatus* and *Naultinus tuberculatus*) found in the north and west South Island are endemic to New Zealand and are classified as Nationally Vulnerable. Detailed information about the ecology, morphology and distribution of these geckos is limited, including what traits are diagnostic, details of their geographic range and it is unknown whether both species hybridise in the wild.

The Denniston and Stockton plateaux in New Zealand's South Island represent a putative contact zone between *N. stellatus* and *N. tuberculatus* and this study investigated whether natural hybridisation occurs between the two species. Ninety-one live geckos were captured, sampled and analysed to study morphological and genetic variation within *N. stellatus* and *N. tuberculatus*. No evidence was found for a contact zone or hybridisation occurring between *N. tuberculatus* and *N. stellatus*; all geckos sampled in this study from Denniston and Stockton plateaux were morphologically and genetically part of *N. tuberculatus*.

The results show significant intraspecific phenotypic variation between populations for each species. Finally, detailed habitat parameters for each individual and documented variation in perch height, both within populations and between sex were recorded.

This information helps conservation practitioners to implement best practice techniques for green gecko conservation management by determining ecotypes and habitat use, as well as population variance. This can prevent the further decline of both genetic variation and risk to species. Specifically, this research aids the mitigation of negative impacts by mining operations and development, the current threat to the Denniston and Stockton plateaux.

## **Developing a breeding facility to meet the changing needs of ectotherm conservation in New Zealand**

Knight J<sup>1</sup>, Laux D<sup>1</sup>

<sup>1</sup>*Wellington Zoo Trust*

The landscape of ectotherm conservation is continually changing, with new species being discovered daily and threats to populations both new and old ever increasing. Conservation bodies must be ready to take a versatile and collaborative approach to ex situ conservation in order to meet the needs of the many. This was the core concept of the redevelopment of our Native ectotherm facility at Wellington Zoo. The facility has been developed to be versatile and adaptable to meet the needs of a broad range of reptiles, amphibians, fish and invertebrates.

The Native ectotherm facility has been built to optimal hygiene standards, with the use of Hydrapanal and hospital grade Degadur Quartz flooring in this facility to ensure the highest standards of husbandry, animal welfare and biosecurity. Also throughout the design process we ensured the build was sustainable from start to finish by ensuring all timber products were FSC certified, via the installation of solar panels onto the roof and by double glazing the entire build to offset and reduce the power consumption of the facility.

Wellington Zoo's Native ectotherm facility has been meticulously designed with versatility in mind; each room operates in isolation with independent heating and cooling. Each room has its own water supply and cleaning facilities, and each room meets containment standards complete with the ability to be placed into individual quarantine if required.

This poster presentation will further outline the methodology used to design and build this bespoke facility with conservation of New Zealand fauna at its heart.

## Ecology and evolution of hot spring frogs

Komaki S<sup>1</sup>

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*Buergeria japonica* is a tree frog widely distributed across Taiwan and Ryukyu Archipelagos of Japan. It is informally called the 'hot spring frogs' as they were reported to be inhabiting hot springs. Notably, *B. japonica* tadpoles from Seranma hot spring on Kuchinoshima Island showed the highest ever recorded water temperatures inhabited by any frog population (46.1°C). Meanwhile, we did observe several dead frogs in the hot spring, and thus, it is doubtful if they intentionally inhabit the hot spring. Here, to clarify whether hot spring frogs prefer inhabiting hot water, we conducted field and behavioral observations. We first investigated the water temperature preference of tadpoles by measuring density of tadpoles in each water pool in the hot spring. Analyses of density data of 54 points showed that the density is higher in cooler water pools. A survey of water temperature preference of 112 adult males for breeding showed they avoid water pools hotter than 37°C. We further performed behavioral observations of oviposition in a laboratory; we put 3 water pools with different temperature (25°C, 30°C, and 35°C) in a cage in which adult male and female frogs were kept for several days; then, we observed 7 oviposition, in which 5 were in a 25°C water pool, 2 were in 30°C, and none in 35°C. Throughout these observations and experiments, any preferences of frogs for hotter water were shown. Our results suggested that frogs even in a hot spring population avoid hot water and tend to utilize cooler water available nearby.

**Different breeding strategy of Korean *Hynobius* salamander according to environments**

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Breeding of organisms is greatly influenced by the environment. However, when the environment changes, organisms adapt to the changes and adopts different breeding strategies. Salamanders in the genus *Hynobius* breed in flowing water such as valley, and lay eggs attaching to substrates in the water. Recently, the salamanders in Oenalo-do, South Korea, were confirmed not to attach their eggs. We investigated the breeding strategy of Korean *Hynobius* salamanders based on the hypothesis "the breeding strategy of salamanders may vary according to the environment". As results, salamanders were attached eggs in 57.4% (n=156) and unattached in 42.6% (n=116) ( $P < 0.001$ ). 48.4% (n=132) of breeding sites were flowing water and other 51.6% (n=141) were standing water ( $P > 0.05$ ). By breeding site types, the channel was the highest with 36.3% (n = 99, attached eggs: 70 > unattached eggs: 29) and rice paddy 32.2% (n=88, 26<64), valley 18.7% (n=51, 40>11) and pond 12.8% (n=35, 18>17) in that order. The eggs tended to be attached more in flowing water ( $r=361$ ,  $P < 0.001$ ). In this study, we confirmed that Korean *Hynobius* salamanders unattached their eggs at a relatively high rate. Attaching is a strategy to prevent eggs from being washed away by flowing water. Therefore, there might be no need to attach eggs in standing water. we presume breeding was more frequent in artificial environments, due to the advantage of maintaining water. Because of the advantages such as water retention, absence of competitors, and heat energy by direct sunlight, many breeding would have been made in rice paddy.

## **Implementation of project “Center of excellence - Cerovačke caves; sustainable management of natural heritage and karst underground” in Croatia**

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The project “Center of excellence - Cerovačke caves; sustainable management of natural heritage and karst underground” was submitted for Competitiveness and Cohesion operational programme 2014-2020. The main goal of the project is to improve sustainable use of natural heritage, primarily in Nature park Velebit in Croatia with focus on Cerovačke caves (Upper and Lower Cerovačke caves). The main problems that were identified that these show-caves are located in undeveloped Lika region, near the town of Gračac, which has poor tourist offer in general, and that the lack of proper long-term development plan for the region and devastation of show-caves. The core activities of the project involve development of a new “state of the art” regional scientific/educational center and revitalization of show-caves Cerovačke caves. To be able to revitalize Cerovačke caves and secure long-term sustainability, various activities were planned to enrich the tourist offer of the region. Aside from the show-caves and educational and hiking trails around the caves, big part of the planned activities focus on education programmes and scientific explorations (schools in nature, trainings for cavers, various workshops and courses, on-site laboratory...) and creation of strong network of potential users from many communities - public, caving, scientific as well as public or private institutions that can use these facilities and programmes. To achieve sustainable use of the caves and the surrounding area, modern solutions in IT visitor management will be implemented, monitoring of tourist impact will be done and newest technical solutions for show-cave inventory will be used. The start of project implementation is planned for April 2018 and duration of the project is 3.5 years.

### Developmental studies of retina differentiation in brown anole (*Anolis sagrei*) embryos

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Native to Cuba and the Bahamas brown anoles (*Anolis sagrei*) lizards are often abundant in suburban or even urban areas. The aim of this study was to investigate development of the retina of brown anole using transmission electron microscopy. The retina of brown anole embryos - diurnally active lizard, started to differentiate just after egg laying. During the first developmental stages, the prospective retina was composed of a pseudo-stratified columnar epithelium with no apparent morphological signs of differentiated neurons. It had a contact with the presumptive retinal pigment epithelium. Photoreceptor cells started to differentiate about a half of developmental period where cilia of presumptive outer segment, lipid droplets, ellipsoid and paraboloid of inner segment were found for the first time. Differentiation of ellipsoid was associated with accumulation of mitochondria formation that undergo various modifications but paraboloid formation was associated with glycogen accumulation. Two types of paraboloid were found. One consisted of granules of glycogen and the other contained tubular membranes continuous with the rough endoplasmic reticulum. At the end of developmental period thin rim of cytoplasm of inner segment formed synaptic pedicle. Müller cells were only one glial cells of developing retina of brown anole were identified by dark cytoplasm, intermediate filaments and glycogen particles. The final differentiation of retina in brown anole occurred after hatching.



### Sex chromosome introgression between two closely related Japanese brown frog species

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The sex chromosomes are undifferentiated (still homomorphic in both sexes) in a majority of amphibians (96% species), while the remaining 4% species show differentiation of heteromorphic sex chromosomes, which occurred during their speciation or geographic differentiation within a single species. This indicates that amphibians are good materials to study on the mechanisms of primary sex chromosome evolution. The Japanese brown frog, *Rana sakuraii*, is genetically divided into two geographic groups in the East and West regions. The former East group has ♀XX-♂XY type of differentiated sex chromosomes, while the West group has never been examined yet. Then, to find out the evolutionary mechanisms of the sex chromosomes in *Rana sakuraii*, we examined and compared the chromosomes of the two groups and then with those of the closely related, ancestral species, *Rana tagoi*, which is distributed sympatrically with the former species. As a result, the West group was found to show undifferentiated, homomorphic sex chromosomes, suggesting that the sex chromosomes evolved morphologically and independently in the East group. Interestingly, it was also found that *R. tagoi*, which is distributed sympatrically with *R. sakuraii* in the East group, had the same differentiated sex chromosomes. Thus, combined with the previous studies on mitochondrial and nuclear genomes of these two species, we conclude that the differentiated, heteromorphic sex chromosomes in the East group of *R. sakuraii* was introduced from the populations of *Rana tagoi* through interspecific hybridization that occurred in the past.

## The impacts of environmental factors on the breeding phenology of *Hynobius* species

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Climate change, represented by global warming, affects the diversity of biological communities and the biological season. Thus, understanding the animal's response to changes in environmental factors can predict the impact of climate change. Especially, amphibian is a thermophilic animal that can breathe through the skin and use both land and water as habitats. It is sensitive to environmental change and is recognized as an indicator species of climate change. This study was conducted to investigate how the breeding activities of domestic *Hynobius* species respond to environmental factors. We set up 27 research sites from 2016 to 2019, and conducted visual surveys at least six times a week from the first week of emergence to record the presence of adults, number of eggs, and larvae. At each survey site, environmental factors were recorded. We set the first appearance date and spawning climax date with the number of eggs. The yearly difference between the first appearance date and the spawning climax date and analyzed which environmental factors were related. As a result, the appearance of *Hynobius* species were tended to be delayed each year, and the breeding activity was affected by various environmental factors. These results show that direct or indirect effects of climate change on biological season related to *Hynobius* species breeding activity. It can also be used as a key resource for estimating past amphibian breeding activity using current climate data and forecasting future breeding activity by applying climate change model.

**Armoured with skin and bone: The integumentary morphology of the Antsingy leaf chameleon *Brookesia perarmata***

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Madagascar's ground-dwelling leaf chameleons (Brookesiinae, including the genera *Brookesia* and *Palleon*) form the sister taxon to all other chameleons (i.e. the Chamaeleoninae) and can readily be recognised by a number of unusual features. Morphologically, most species of the genus *Brookesia* stand out due to the presence of a peculiar "Rückensäge", a spiky row of dorsolateral projections of the vertebral column and an aberrant vertebral structure that has been subject to several osteological studies. In addition to the pronounced Rückensäge, the Antsingy leaf chameleon *B. perarmata* exhibits conspicuous, acuminate tubercle scales on the lateral flanks and extremities, potentially indicative of some form of integumentary armour. Such structures are not known for any other chamaeleonid and despite an appreciable interest in chameleon integumentary research in general, so far it was completely unknown what these tubercles truly are.  $\mu$ CT imaging and various histological approaches revealed that the tubercle scales consist of osseous, multi-cusped cores embedded within the dermis, and that they consequently can be interpreted as osteoderms. To the best of our knowledge, this is the first record of osteoderms in the Chamaeleonidae. In some aspects of their tissue composition and location within the dermis, the osteoderms of *B. perarmata* differ from those known for lepidosaurians so far, but rather are somewhat reminiscent of crocodylian osteoderms. Therefore, our histological data also contribute to the knowledge about the structural diversity of lepidosaurian dermal armour in general.

**Expression of calcium binding proteins in the oviducts of two lizards with different reproductive modes**

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Calbindin-D28k is one of the most important calcium binding proteins (CaBPs) and plays significant roles in Ca<sup>+</sup> transport, protecting the cells from high concentrations of Ca<sup>+</sup> or from cellular degradation via apoptosis. However, the characterization of CaBP-D28k gene and its expression in germ cells in lizards remain poorly understood. We cloned the CaBP-D28k gene from *Sphenomorphus indicus* (viviparous species) and *S. incognitus* (oviparous species), then we examined the mRNA expression in oviducts of these two lizards at three different states (preovulation, pregnant and postpartum). Phylogenetic analysis revealed that *S. indicus* and *S. incognitus* were grouped together, and they did not match sequence particularly close to them, but with gecko, anole lizard attributed to the branch of squamata. The expression of CaBP-D28k gene is consistent in preovulation, pregnant and postpartum of these two lizards, with lower expression during preovulation, and significantly up-regulated during pregnant, then was significantly down-regulated in postpartum. This result suggested that the CaBP-D28k gene may be involved in the transport of Ca<sup>+</sup> from mother to embryo during maternal pregnancy. In addition, the developmental pattern of CaBP-D28k gene expression in oviparous lizards is similar to that of viviparous lizards, which indicated that calcium transport mechanisms and their regulation are independent of reproductive mode. This study is the first time to analyze the characterization of the cDNA sequences of CaBP-D28k in *S. indicus* and *S. incognitus* and explore this gene expression between oviparous and viviparous lizards, providing evidence for further research of viviparity evolution from a molecular biology perspective.

## The phylogeny and diversity of *Gekko* genus in China

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The Gekkonid lizard in China, possessing high diversity and also include the China-specific species, is a good material to study the formation of biodiversity. However, most of the research in the past did not explain the relationships of these species between China area and Southeast Asia area separately, neither of them focusing on the relationship of Gekkonidae species between China and Southeast Asia. Furthermore, they do not include enough samples and sequences from China and fail to reveal the relationship inside Chinese Gekkonidae species. Our aim is to conduct the field work in wide area in China and reconstruct the phylogeny tree of China Gekkonidae species. Our research used the ND2, CytB and 16S genes to reconstruct the phylogeny tree and we found that most of our results about the relationships between genes correspond to the articles in the past. Apart from that, our research also revealed that the diversity of *Hemiphyllodactylus* genus has been underestimated and there exist the species delimitation in the *Gekko* genus.

**Adjusted energy allocation and condition-dependent call strategies of *Rhacophorus prasinatus* under noise interference**

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Condition-dependent strategies have been reported in various fields of behavioral ecology, but have never been studied under the perspective of acoustic communication. In this study, we aimed to test the energy allocation and acoustic plasticity of a rhacophorid treefrog (*Rhacophorus prasinatus*) under noise interference, and compared the strategies between high-quality and low-quality males. We found that the frogs showed strongest avoidance to the medium-frequency noise that overlaps with their own signal; this noise also stopped the frogs from normal aggregating behavior. When broadcasting synthetic noise–silence cycles, males immediately adjusted their call tempo by using the silent gap to avoid signal overlap. Furthermore, males also showed a regulation ability, where they moved up their call starting time in the short silence gap, which helped them to deliver more notes within a limited period of time. When broadcasting continuous noise, high-quality males maintained the original, intense call rates, which might be an honest signal of their high quality. In contrast, low-quality males ceased calling, possibly to serve as satellite males. The opposite strategies adopted by high-quality and low-quality males indicated that the strategy utilization could be condition-dependent. The final strategy seen in the wild is a compromise among several contradictory factors, such niche competitor, noise interference, and the frog's own body condition.

**Mobile freshwater reefs: ectoparasites and ectocomensal epibionts on the bottom-dwelling freshwater turtle *Hydromedusa tectifera***

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The present work describes the community of ectoparasites (EP) and ectocomensal epibionts (EE) that inhabits on the chelid turtle *Hydromedusa tectifera* from an urban-suburban stream (Rodríguez stream, La Plata City, Buenos Aires province, Argentina). We also test variations in the taxa composition and abundance of EP and EE by comparing two stream sections, one mid-section impacted by urban pollution (S1) and other rural placed near stream headwaters (S2).

Turtles were sampled manually during summer (2018), winter and spring (2019), sexed and sized (straight carapace length and weight) and all EP and EE were removed at the moment of the capture and carried to laboratory (Ethanol 70%). Importance of each EP-EE was assessed using the IRI index. Significant variations on the numeric frequency of fundamental EP-EE IRI taxon types among stream sites and sex of the turtles were analyzed using three non parametric ANOVA (Kruskal Wallis).

We caught 65 turtles and found 1251 invertebrates that conformed a 9 taxon types EP-EE assemblage. According the IRI, the relevant EP-EE types were immature quironomids, amphipods and leeches. We detected significant differences for amphipods ( $H= 21.36$ ,  $p= 0.0004$ ,  $df= 5$ ), quironomids ( $H= 12.77$ ,  $p= 0.02$ ,  $df= 5$ ) and leeches ( $H= 8.68$ ;  $p= 0.02$ ,  $df= 5$ ). Post-hoc Dunn's test showed that (1) numeric frequency of chironomids is higher in S2-males than in both sexes from S1, (2) S1 females and juveniles have fewer amphipods than S2-males; and (3) S2 females and juveniles presented fewer leeches than S1-females.

**The diversity and biogeography of Music frogs (Anura: Ranidae: *Nidirana*)**

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The taxonomic treatment of the music frogs within ranid genus *Nidirana* was controversial in a long time. The most recent contribution to the phylogeny of this genus reconsidered it as a distinct genus, based on comprehensive evidences of morphology, mitochondrial phylogeny, bioacoustics and biogeography. Eight species are currently recognized from subtropical eastern and southeastern Asia. Nevertheless, the interspecific relationship of these frogs remains unresolved. The delimitations of several particular species are controversial. In the current work, on the basis of extensive sampling, we reconstruct the phylogeny of this genus. The result indicates that the current records of *N. adenopleura* and *N. pleuraden* both are species complex composed of multiple cryptic species. Bioacoustic and morphological analyses also support the phylogenetic results. In addition, the distributions of each species are clarified.



**Conservation genomics in the endangered Australian pygmy bluetongue skink, *Tiliqua adelaidensis***

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The Australian pygmy bluetongue skink (*Tiliqua adelaidensis*), is an endangered species endemic only to South Australia. *T. adelaidensis* exists in highly fragmented habitat and has no capacity for long distance dispersal. Long term conservation of this species will rely on assessments of population health, the species' own ability to rapidly adapt to changing conditions, and continued research into translocation efforts. The objective of this project is to identify areas of the genome that may allow adaptation to new environmental challenges. RNA-seq was used to measure and compare gene expression between samples collected at the beginning and end of the dry season. Kidneys from eight individuals were sequenced to ensure the capture of genes associated with surviving dry periods, and so that results can potentially be used as reference sequences for concurrent studies. Whole long-read Iso-Seq transcripts have also been sequenced to compare transcripts and ensure the accuracy of assembly methods in the absence of a closely related genome for this species, and assist in gene annotation. This study measures gene expression at the individual level, reflective of a pygmy bluetongue's ability to adapt to changing environmental conditions in the short term.

## Unravelling the complex taxonomy, systematics and biogeography of Asian horned frogs of Northeast India

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The Asian horned frog genus, *Megophrys*, is poorly studied, despite having an extensive distribution from the southern Himalayas to eastern China, and south to Indonesia. The conservative morphology exhibited by many species in this genus has led to widespread misidentifications in the literature, producing a very confused taxonomy. Recent molecular studies have dramatically helped to unravel the complexity of the systematics in this group and highlighted extensive hidden species diversity. However, complicated taxonomic issues relating to the identity of historically named species have hindered comprehensive revision.

Here, we aimed to resolve nomenclatural and taxonomic issues associated with the two earliest named species from the subgenus *Xenophrys*, *Megophrys (X.) monticola* and *M. (X.) major*, both described from Northeast India and thought to also be widely distributed in Southeast Asia. Dense sampling across most of Northeast India, a detailed study of historical specimens, and phylogenetic analyses permitted the redescription of these two species and the identification and description of several new species in the region. The taxonomic revision of Northeast Indian *Megophrys* has provided novel insights into the biogeography of these frogs in the Eastern Himalayas, highlighted areas of high species diversity, sympatry and even syntopy between closely related species, and revealed extensive misidentifications in the literature. This work has prompted reassessments of species distributions that have resulted in both considerable range restrictions, and substantial range extensions.

**Morphological variation, geographic distributions, and taxonomic boundaries of map turtles (*Graptemys*) in Kansas, U.S.A**

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Kansas lies at the western extent of the distributions of four map turtle taxa native to temperate North America—the Northern (*Graptemys geographica*), Ouachita (*G. ouachitensis*), Northern False (*G. p. pseudogeographica*), and Mississippi (*G. p. kohnii*). Occurrence and geographic distributions of map turtles in Kansas are poorly documented because many museum specimens lack diagnostic features (e.g., many are only shells and lack soft parts), authorities disagree on diagnostic characteristics, and species limits are in dispute. Being morphologically similar, these taxa can be confused with one another in areas of distributional overlap. Currently, in the herpetological literature for the region, the Ouachita, Northern False, and Mississippi map turtles are treated as an unresolved species complex. Our goal is to document and analyze the geographic distribution of morphological variation related to criteria for diagnosing these taxa. To obtain reliable locality records for Kansas, we are conducting visual surveys (by telescope) and catching turtles with baited traps. We are also reviewing historic records of these species' occurrence by examining voucher specimens in museum collections. In 2017--2018, we captured 110 map turtles, all of which were easily assigned to taxa, with none having ambiguous morphological features used in taxonomic diagnoses. On the basis of those morphological characteristics, we have captured 7 Northern, 13 Northern False, 73 Ouachita, and 16 Mississippi map turtles thus far. To further investigate species limits, we will conduct genetic analyses of tissue samples from captured individuals.

### Microhabitat selection of *Sinonatrix percarinata* in a flood pulsing montane lake in northeastern Taiwan

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Compared to the freshwater wetland system of rivers of plains, flood pulsing is relative rarely in the montane lakes, most likely due to the smaller sizes of the catchment area. Typhoons are large tropical storms that form in the Northwestern Pacific Basin and irregularly impact the ecosystem dynamics of eastern Taiwan. Typhoons that follow certain landfall routes have been determined to be the primary driving force behind flood pulsing at Mystery Lake, located in Yilan County, northeastern Taiwan. From February 2018 to January 2019, a monthly mark and recapture population study of *Sinonatrix percarinata* was conducted at Mystery Lake. The snakes were collected along five transect lines, using 64 modified floating funnel traps, that were set along three axes of the lake (transect I-III), at the inlet canal (transect IV) and in the creek (transect V). Concurrent biotic factors (e.g. the number of tadpoles, loaches, leeches, etc.) in the traps were counted and record for further analysis as well. A drone was utilized to record the vegetation cover ratio of each trapping site once a month. The results indicated that *S. percarinata* primarily coincided with *Sparganium fallax* and *Schoenoplectus mucronatus robustus* vegetation. Compared to the other four transects, *S. percarinata* was more often recorded along the transect at the inlet canal. The microhabitat characteristics and the correlations with biotic factors will describe in the poster.

**Can the thermal characteristics of *Trimeresurus stejnegeri* be determined from long-term field observation data?**

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An ongoing long-term study into the population dynamics of an ambush predator, *Trimeresurus stejnegeri*, and coexisting snake species in Yenwen Experimental Forest of National Ilan University in the northeast of Taiwan, was initiated in June 2010. Visual encounter surveys are undertaken at fixed times of the day, irrespective of the time of year or the weather, along an established transect to avoid possible researcher-related biases. Preliminary data analyses suggested a normal distribution pattern along the thermal gradient, with a peak at the 22.1-24°C interval, which is close to what has earlier been determined to be the preferred temperature of *T. stejnegeri*.

This result prompted the questions, can the recording of the ambient temperature at the time when a nocturnal snake is encountered in the field be used to determine the thermal characteristics of the species and assess possible other related issues; and if so, how much data is needed to meet the minimum requirements?

To date, 465 inventory nights and 743 encounters in which the air temperature and the locality of the snake were noted and have been recorded. The results from the analyses of this data will be compared with those from controlled laboratory studies, and discussed in the poster.

**The demography of *Sinonatrix percarinata* following extreme climatic events in a montane pond, northeastern Taiwan**

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From July 2010-October 2011 a preliminary study was undertaken at Lunpi, a small closed montane pond in Yilan County, northeast Taiwan, to establish basic data for long-term monitoring of the ecological dynamics and fauna and flora succession in this wetland ecosystem due to climate change. Three types of funnel traps were set along four axes of the pond to sample fauna utilizing the littoral, limnetic and benthic zones to determine their spatial occurrence. A primary focus was on the population of the predominant water snake, *Sinonatrix percarinata*. Every captured snake was examined to determine its body condition, measured and then injected with a PIT for future identification, after which it was released back into the wild.

The methodology of the monitoring involves studies undertaken at 5-year intervals, so the second study took place from October 2015 to August 2016. This period experienced dramatic fluctuations in temperature and precipitation, which caused a decline in the original dominant plant species, *Brasenia schreberi* and increase in *Utricularia gibba*. In addition, the abundance of *S. percarinata* in the pond greatly declined. In September 2016, typhoon Megi had a dramatic impact on the pond, changing the vegetation distribution and the landscape. As a result, the study was extended to November 2017 to examine the resilience of the wetland ecosystem and record the changes caused by this event. In this poster, we will compare and discuss the demographic and spatial differences of *S. percarinata* from after the two study periods.

## Postcranial skeleton of a snake from the Mealla Formation (Paleocene) of Northwestern Argentina

Triviño L<sup>1</sup>

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Although in South America snakes are recorded from the Upper Cretaceous, it is during the Paleogene that their diversity becomes more evident with Bolivia, Peru, Colombia, Ecuador, Brazil and Argentina. Particularly for Argentina, the majority of the squamates (Reptilia: Squamata) of the Paleogene are represented by snakes from Patagonian localities. In addition to several indeterminate forms, four genres are recognized: *Madtsoia*, *Chubutophis*, *Waincophis* and *Boa*. Apart from these Patagonian records, the Argentine Northwest (NOA) has provided scaly remains in the Lower Lumbreira Formation, including a Tupinambino lizard and a snake still unpublished. The record of the NOA is increased with the presentation here of a postcranial skeleton of a snake from the Mealla Formation (Mina Aguilar, Province of Jujuy, Argentina). Most of the vertebrae are from the middle of the trunk; they are large and robust, shorter than wide, and wider than high. The absence of prezygapophyseal process and the presence of parazygantral and paracotyler foramina suggest affinities with the Family Madtsoiidae. This family constitutes an extinct group of continental snakes of fundamentally Gondwanic distribution. In South America the presence of madtsoideos is documented from the Cretaceous to the Oligocene, being in Argentina only in Patagonia. The presence of this madtsoideo in the NOA improve the South American record of the group and its distribution during the Paleogene.

**Neuroanatomy of Philodryas (Dipsadidae): 3D reconstruction vs dissection****Mardones Gutiérrez M<sup>1</sup>**<sup>1</sup>*National University Of La Plata*

The surface morphology of the brain of the Argentinean snakes *Philodryas aestiva* and *P. patagoniensis* (Dipsadidae) is analyzed using dissection and computed microtomography, respectively. In dorsal view, fore-, mid- and hind-brains are placed consecutively and with decreasing development. In dorsal view, the dissection indicates that the brain fills the endocranial cavity (100%), but, in lateral view, there are two spaces located ventrally to the olfactory lobes and laterally to the cerebellum. Such spaces are not reflected in the digital endocranial cast, information of interest for paleoneurologists. Olfactory tracts leave their impression on the ventral side of the frontals projecting large olfactory lobes anteriorly. The vomeronasal nerve projects towards the palate, exiting through the vomeronasal fenestra. The optic tract exits through a foramen located between the frontal and the parietal on each side. Oculomotor, trigeminal, abducens and facial nerves leave the braincase through foramina bounded by the prootic. The trigeminal has two internal foramina, the anterior is for the ophthalmic branch, whereas the posterior (and larger one) externally presents the semilunar ganglion from which the maxillary and mandibular branches diverge ventrally. Posterior the trigeminal foramen, the hyomandibular branch of facial nerve exits through a single foramen in the prootic. The palatine branch enters the skull again through the posterior foramen of the vidian canal, in the basisphenoid. In the posterior area of the skull, exiting through a single foramen near to the occipital condyle, there are three nerve fibers surrounded by connective tissue. They are the glossopharyngeal, vagus and hypoglossal nerves.



### It's Getting Hot in Here: Potential Effects of Climate Change on Andean Lizards

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Climate change is one of the greatest threats to Lepidosauria across the globe. *Proctoporus unsaacae* and *P. sucullucu* are cold-adapted thermoconforming lizard species native to the high Andes mountains, where increasing their elevation to escape temperature increases is impossible. Referencing predicted temperature increases for the Andean region of Peru in the 21st century, this study aimed to better understand the thermal tolerances of both species. We hypothesized that both species would fail to maintain normal function above 32 °C, which is near the maximum temperatures these species encounter in their microhabitats. We tested 61 *Proctoporus* lizards to determine critical thermal maxima (CT max) by slowly increasing the temperature of a water bath until they were unable to maintain normal bodily functions. We also measured microhabitat daily fluctuations to calculate warming tolerance. Despite our predictions, the species had much higher thermal tolerances than expected, with a mean CT max of over 38 °C, and with some lizards exceeding 41 °C. This suggests that even with rising global temperatures, *P. unsaacae* and *P. sucullucu* will not reach temperatures too high to survive, though their function may be decreased as microhabitat temperatures climb. Additional studies are needed to determine if high temperatures decrease reproduction or fitness in these species.

**Steroidogenic activity in gonad-adrenal-mesonephros complex during transitory pseudohermaphroditism stage in *Plestiodon copei* (Squamata: Scincidae)**

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The sexual development of saurians follows a similar pattern to that described for other amniotes, however recent reports shown that some species show heterochrony between gonadal differentiation and dimorphic development of external genitalia. In such species, the newborn females show hemipenes at and clitoris formation occurs during posnatal life. Here, we describe and analyze the steroidogenic capacity of gonad-adrenal-mesonephros complex and determine the presence of  $\alpha$ -oestrogen and androgens receptors in the hemipenes in both sexes during transitory pseudohermaphroditism stage in the Mexican skink *Plestiodon copei*. The new-born of *P. copei* do not exhibit body sexual dimorphism; however both sexes show hemipenes and conserve the mesonephros. The females have oviducts and ovaries well differentiated whereas the males show testicles with seminiferous cords. In females the hemipenes regression and clitoris formation occurs during early postnatal life. We found that the gonads and mesonephros in both sexes do not show steroidogenic activity. However, the adrenal glands in both males and females were positive to  $\Delta^5-4-3\beta$ -hidroxi steroid dehidrogenase but negative to 17ceto reductase. Moreover, we found that the hemipenes of new born lack receptors androgen and estrogens. This situation show that exist a delayed in somatic cell differentiation gonadal and in receptors expression in the hemipenes of newborns of *P. copei*. We thought that the external genitalia should be considered as protohemipene and protohemiclitoris in males and females newborns, respectively, of this specie.

**Evaluating the effects of environmental factors on the anuran-*Batrachochytrium dendrobatidis* system in the US Midwest**

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The effects of wildlife diseases are moderated by environmental conditions like temperature, ultraviolet (UV) radiation, and food availability, which influence hosts, pathogens, and their interactions. The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has been implicated in global amphibian declines, yet its effects vary between geographic regions and hosts. We evaluated the influence of environmental factors on this system in experimental laboratory studies and in a field survey in the United States Midwest. Midwestern anurans can be infected by *Bd* and experience decreased terrestrial growth and overwintering survival in a laboratory setting, yet no *Bd*-associated population effects have been observed in this region. In the lab, we found that ambient UV moderated the effects of *Bd* on American toads (*Anaxyrus americanus*) by decreasing the quantity of *Bd* to which they were exposed. Metamorphs exposed to the *Bd* pathogen experienced significantly reduced terrestrial growth and overwintering survival at temperatures on either extreme of the *Bd* thermal optima. We investigated the hypothesis that decreased feeding behavior in infected individuals may be causing reduced terrestrial growth, and found that infected individuals consumed more food and worked harder than uninfected individuals to find food hiding under refugia. In a field study, we surveyed a series of ponds that span the conditions tested in the laboratory to examine factors that predict *Bd* on anurans across five months. These results could inform wildlife and conservation management decisions aimed towards decreasing exposure to this pathogen and help predict conditions that lead to susceptibility to *Bd* and disease development.

### Physiology and climate change implications on thermal biology of an endemic teiid lizard from Brazil

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The threatened lizard *Glaucomastix littoralis* occurs in only four restinga habitats of the Brazilian coast. Due to its restricted distribution, the effects of habitat degradation associated with changes in climate are especially harmful for this species. To estimate local or even global extinction, we analyzed the ability of *G. littoralis* to cope with current climate changes by evaluating its thermal biology. We calculated thermal performance curves, hours of activity restriction (Hr) based on its thermal preference and three indexes evaluating the efficacy of thermoregulation: accuracy of body temperature regulation (db), thermal quality of the environment (de) and effectiveness of thermoregulation (E). The specie' optimal locomotor performance (41.8oC) occurs above maximum voluntary temperature (VTmax) (40oC) and above body temperature in activity Tb (38+2.1oC, N=71). The population from Jurubatiba presented the best accuracy of termorregulation with Tb inside the set-point range (Tset) limits for males, females and juveniles. At Maricá restinga habitat, males had accuracy outside Tset limits (db=-0.1) and at Grussaí the population had the worst accuracy value, with both sexes and juveniles showing a Tb outside the limits of Tset, especially females, suggesting that the environment is not thermally ideal. The future predictions were based on both optimistic and realistic scenarios of greenhouse gases emissions and indicated a lack of thermal suitability of up to 69.4%. The results demonstrate that *G. littoralis* is already experiencing negative effects of climate change, which associated with high rates of habitat loss, indicate a high probability of extinction of the species.

## NEWTRAP - A camera trap for underwater newt studies and population estimates

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Methods commonly used to estimate newt population size rely on capture-recapture approaches (using dip net or funnel trap) requiring intensive field effort, handling individuals and disturbing habitat. Therefore, population size estimates are difficult to obtain on the long-term and/or at large scale. We developed an underwater camera trap (NEWTRAP; patent LU93388) automating the production of ventral images (colour or infrared) without newt handling. The motion of individuals triggers the recording of images and/or videos with standardised newt position, background and lighting, expediting visual image processing. The NEWTRAP is configured to work round the clock and has currently a battery life of up to five days. A dedicated web application (NEWTRAP Manager) supports the management of metadata and the annotation of images and videos. Based on linear SVM (Support-Vector Machine) and HOG (Histogram of Oriented Gradients) features, raw images are classified by newt species, and ventral areas of *T. cristatus* are extracted to be submitted to an automated photo-identifier software such as AMPHIDENT.

NEWTRAP ventral images display criteria to determine species, stage of development, gender and size of the four native newt species of Northwestern Europe (*T. cristatus*, *Ichthyosaura alpestris*, *Lissotriton vulgaris* and *L. helveticus*).

By automating the observation of individuals, NEWTRAP supports large dataset acquisition, with high temporal resolution and reducing labour cost. A single observer covers a larger number of ponds by day without spending time in newt handling. A retail version should be available by early spring 2020. The study is cofounded by the Luxembourg National Research Fund.

***Batrachochytrium salamandrivorans (Bsal)* chytridiomycosis it's not just for salamanders!**

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*Batrachochytrium salamandrivorans (Bsal)* is a chytrid fungus that infects amphibians, causing skin lesions and eventually death. To date, it has been thought that *Bsal* only leads to asymptomatic carrier states in frog species and does not cause disease as it does in susceptible salamander species. Therefore, we hypothesized that anurans would not develop disease when challenged with concentrations of *Bsal* that result in disease in salamanders. To test this hypothesis, we exposed invasive Cuban treefrogs (*Osteopilus septentrionalis*) to either a mock inoculation of autoclaved dechlorinated water or one of four concentrations of *Bsal* zoospores  $5 \times 10^3$ ,  $5 \times 10^4$ ,  $5 \times 10^5$ , or  $5 \times 10^6$  via water bath (n = 4 per zoospore dose). Frogs were swabbed every six days during water-change events to document the onset and progression of infection. Frogs exposed to  $5 \times 10^6$  zoospores tested positive for *Bsal* using qPCR starting four days post-exposure. Beginning two weeks post exposure, these frogs began showing signs consistent with *Bsal* chytridiomycosis, including excessive shedding, increased pigmentation, and petechia. Histopathology was performed on eight animals; two of which died naturally. Multiple erosive crater-like epidermal lesions were randomly distributed throughout the body and varied from superficial to full thickness epidermis. Lesions consisted of focal epidermal necrosis with numerous intralesional *Bsal* thalli that were evident when stained with H&E. This challenge experiment emphasizes that the conservation threat of *Bsal* may extend beyond salamander species.

**Clinical pathology of *Bsal chytridiomycosis*: hematological, biochemical, and serum protein analyses of infected *Taricha granulosa***

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<sup>1</sup>University Of Tennessee, <sup>2</sup>University of Miami

*Batrachochytrium salamandrivorans* (*Bsal*) is a recently discovered pathogen that is of global concern because of its potential to cause high mortality in amphibians, especially salamander species. Although little has been reported on the pathophysiological effects of *Bsal*, studies have made headway in understanding the pathogenesis of *B. dendrobatidis* (*Bd*), a similar fungus that has led to global amphibian population declines throughout the world. Studies found that electrolyte losses occur in amphibians infected with *Bd* from loss of osmoregulation through extensive skin pathology, and it is hypothesized that changes in electrolyte concentrations lead to paralysis and cardiac arrest. In our study, we hypothesize that species susceptible to *Bsal chytridiomycosis* would have similar losses in electrolytes, because like *Bd*, *Bsal* damages the epidermis and may affect skin osmoregulation. To test this hypothesis, we collected blood from the hearts of a larger susceptible species, *Taricha granulosa*, at necropsy using heparinized capillary tubes. A blood smear was prepared, and a portion of whole blood was added to Natt Herrick's solution for total blood cell counts. The remaining blood was centrifuged and plasma was collected for blood chemistry profiles and protein electrophoresis. Initial biochemical results revealed decreases in sodium, chloride, and potassium in clinically affected individuals (5x10<sup>6</sup> dose). Moreover, protein electrophoresis revealed changes in protein concentrations that suggest the presence of inflammation and antibody production. By understanding the pathogenesis of *Bsal*, we aim to gain insight in developing treatment options and opportunities to mitigate and prevent spread of disease.

**An account of true sea-snakes of sunderban biosphere reserve and adjoining coasts of west bengal****Mondal S<sup>1</sup>**<sup>1</sup>*Zoological Survey Of India*

Sunderban Biosphere Reserve (SBR), the World Heritage Site & Ramsar Site, represents world's largest coastal wetland with mangroves ecosystem in the delta of rivers Ganga, Brahmaputra and Meghna between India and Bangladesh while India shares around 40% of total area. West Bengal shares entire portion of Indian SBR and represents only 2.09% coastline of India but harbors enriched biodiversity spreading across wide range of habitats viz. deltatic islands, estuaries, network of creeks, parallel beach ridges, mudflats, sandy plains, dunes and sandflats. The true sea snakes belonging to Subfamily Hydrophiinae are significantly important but their biological and ecological information are scanty from Indo-Pacific regions especially from Indian coasts. This group of elapids are least studied in the coastal areas of SBR as well as adjoining coastal areas of West Bengal. A comprehensive study was carried out on the National Zoological Collections of Indian Museum to increase knowledge about this group of marine reptiles. A total of eleven species were recorded during this study including nine species under the genus *Hydrophis* and two species belonging to the genus *Microcephalophis* along with two type specimens *Hydrophis fasciatus* and *H. nigrocinctus* mentioning SBR as type locality. The study also revealed that the sampling occurrence of *H. schistosus* was maximum with 47.05% followed by *H. obscurus* with 13.72% while *H. viperinus*, *H. platurus*, *H. mamillaris* and *H. nigrocinctus* were with the sampling occurrence of only 0.19% respectively. Comprehensive in-situ studies are required to validate the species diversity of Hydrophiinae from West Bengal coastline including SBR.



## **Rapid Action Survey (RAS) on risk assessment of Snakebite in rural villages of West Bengal**

**Mondal S<sup>1</sup>**

*<sup>1</sup>Zoological Survey Of India*

Snake-bites are well-known medical emergencies in many parts of the world especially in rural areas. Agricultural workers and children are the mostly affected. Snake-bite is extensively reported among the agriculture-based workers and children and resulted as fatal or with major disabilities. A study was conducted in Kinkarbati village, Hooghly, West Bengal, India for a period of two years. Quantitative and qualitative studies were made on the snakes along with habitat ecology and community-based interview with the local villagers to record the snakebite history and man-snake conflict. The data were documented seasonally viz. pre-monsoon, monsoon and post-monsoon. A total of 605 houses and adjoining agricultural fields were surveyed while the maximum of 45.45% of multiple microhabitats were documented from houses where 46.70% of habitats were inside the houses. It was found that major man-snake conflict was documented during monsoon in agricultural land due to disruption and flooding of snake's microhabitat while majority of siting, conflict and bite was recorded in houses nearer to agricultural land during late monsoonal and initial post-monsoonal season due to the unknowingly creation of microhabitat with the agricultural byproducts by the villagers to use those for their daily livelihood. The rate of illiteracy, believe on traditional healing methods, poor knowledge on venomous and non-venomous snakes, ignorance to use torch, footwear, mosquito-net etc. use to increase the chances of conflict, snakebite and resulted as death. Awareness among the communities is the key to reduce the snakebite, also for the sustainable conservation of these significant biological organism.

### **Hormonal Herps: Stress and Physiology of an Endangered Lizard (*Gambelia sila*)**

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The federally endangered blunt-nosed leopard lizard (*Gambelia sila*) inhabits the rapidly disappearing San Joaquin Desert of California. This population of lizards has a three month-long active season, during which all reproductive activity seems to take place. The reproductive cycles of this relatively under-studied species can be better understood using endocrine profiles (including hormones like testosterone and estradiol) of blood samples taken from adults at different points in the active season. Additionally, other studies involve deployment of radio collars around the lizards' necks to study other aspects of the lizards' biology. However, the extent to which the collars impact the animals is unknown. Sub-lethal effects of radio collars may be detected by examining hormonal evidence of stress. Corticosterone (CORT) is a glucocorticoid hormone and mediator of stress, and high plasma CORT levels may suggest an animal is under some form of physiological stress. At the end of a 2.5-month radiotelemetry project, we collected blood samples from collared and uncollared blunt-nosed leopard lizards. Preliminary results suggest baseline CORT and stress reactivity (the rise in CORT from baseline following an acute stressor) did not differ between radio-collared and uncollared lizards, indicating that wearing radio-collars may not be a stressor to the lizards. Examining these endocrine profiles provides much-needed data on reproductive cycles and suggests that radio-telemetry may not be stressful to blunt-nosed leopard lizards, implying that our field methods for tracking sensitive species do not negatively impact them.

### Species Recovery of the Cinnamon Bush Frog in Singapore

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<sup>1</sup>*National Parks Board*

The Cinnamon Bush Frog (*Nyctixalus pictus*) is a small tree frog that breeds naturally in phytotelms. Arboreal and nocturnal, this species is locally rare in Singapore and is restricted to the nature reserves and the peripheral nature parks. As part of the species recovery programme, translocation of the species was attempted. Tadpoles and/or adults were transferred to two potential suitable sites outside its known distribution and monitored closely. The results indicate that some of these individuals are doing well and are even breeding at the translocated site. The success of this study will contribute to the limited knowledge of conserving these tree frogs and aid in ensuring its future survival.

**New insights on the geographical distribution, ecology, and conservation status of the endemic Salt Marsh Gecko (*Cryptactites peringueyi*)**

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The Salt Marsh Gecko, *Cryptactites peringueyi* (Boulenger, 1910) is an endemic leaf-toed gecko known from two populations in the Eastern Cape, South Africa. In the past, this gecko has caused much confusion and once referred to as “one of the herpetological mysteries of the region”. Since then, our knowledge of the species has grown, in both a molecular and geographical sense. However, through recent surveys our understanding of the species on a geographical level has increased considerably due to new insights into the species ecological preferences. This allowed for surveys of both historical localities along with newly identified sites. Our findings resulted in recording the presence of *C. peringueyi* at three historical sites, and five new localities. These findings resulted in an increase of Extent of Occurrence from 785 km<sup>2</sup> to 1238 km<sup>2</sup>. Data from the preliminary surveys already resulted in a downgrade in conservation status from Critically Endangered to Near Threatened in 2018, with the additional most western record at Eersteriverstrand and the protected locality within Sardinia Bay we further recommend a new listing of Least Concern. The reason being that it occurs over a wider range, has no substantial threats and occurs within several protected areas. This will make *C. peringueyi* only the third species of reptiles globally to undergo a previous listing of Critically Endangered into the Least Concern category; all due to improved knowledge on the species. This data illustrates the importance of fully understanding a species ecology in order to address a species conservation status.

### Living on moving islands: the ecto-parasites of sleepy lizards inhabiting an ecological gradient

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Parasites are major elements of biodiversity and play important roles in ecology by influencing host population dynamics, interspecific competition, and energy flow. Spatial variations in temperature and/or humidity create ecological gradients, which influence the distribution of various species and their parasite communities. These ecological gradients therefore provide natural experiments for how species associations may change with climate perturbations. However, the influences of ecological gradients on the parasite communities of wildlife is poorly understood. This knowledge gap is in part due to limited data on host/parasite associations within different ecosystems. This research forms part of the long-term field surveys on the sleepy lizard (*Tiliqua rugosa*) at a study site near Mt. Mary in the Mid North region of South Australia. It utilizes established transects, which cross a precipitation-induced natural ecological gradient, to study the ecto-parasite (ticks and mite) communities of the lizards that inhabit this locality. Earlier research at the study site focussed on the ticks, *Amblyomma limbatum* and *Bothriocroton hydrosauri* and their interactions with each other as well as their primary host, the sleepy lizard. This study incorporated the other more rarely encountered ecto-parasites that have been observed parasitising sleepy lizards to provide a more comprehensive description of the ecto-parasite community of the lizards. It was found that the ecto-parasite communities differ across the ecological gradient due to climatic and biotic factors.

## Species Richness and Endemism of Reptiles in Mt. Kalatungan, Mindanao, Philippines

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Reptiles are highly vulnerable to anthropogenic disturbances. This study was conducted in the montane forest of Mt. Kalatungan Range where agriculture is slowly expanding from the lowlands to the montane forest. Sampling sites were established in the lower montane forest at 1000-1400 meters above sea level (masl) and at the upper montane forest at 1400-1600 masl. This study was conducted to determine the species richness and endemism of reptiles in Mt. Kalatungan Range using the cruising method. Five reptile species under four families were documented. A 60 % endemism was recorded. The low species richness is attributed to the high elevation and low temperature of the sampling sites. *Rhabdophis auriculata*, an endemic species, was the most abundant species found in the lower montane forest. The high endemism indicates the importance of Mt. Kalatungan Range as habitat of reptiles.

## Lizards get the flu – exploring the shingleback nidovirus in an iconic Australian lizard

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The sleepy lizard (*Tiliqua rugosa*) is an iconic and widely distributed Australian skink that is ecologically well-researched. It has one of the longest and most complete ecological datasets of any reptile in the southern hemisphere (37 years). Recently, a nidovirus from the same family as the SARS virus in humans (Coronaviridae) was characterised within this species, and has been linked to a serious upper respiratory tract infection in *Tiliqua rugosa*. Treated animals have a very high survival rate (84%), however it is thought that in untreated wild individuals there is a significantly higher mortality rate. Very little is known about this nidovirus, including its distribution. Most infected *T. rugosa* are reported in Western Australia, however a small number of sick animals have been reported in South Australia. Due to this pattern of infection, and the continuous species distribution across states, it is crucial to assess this virus' distribution. I am developing an antibody test (ELISA) to determine whether an individual has previously contracted this specific virus. I will be using reverse transcription real-time PCR to determine individuals that are currently infected. These data will generate a geographic distribution of where the virus is active, and where it has been previously, across southern Australia. The PhD project also focuses on differential gene expression between infected and non-infected individuals, to understand the host's genetic immune response to this virus.

***Glandirana rugosa*: The frogs that carry dual sex determination systems within conspecies**

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<sup>1</sup>Waseda University

In general, each vertebrate species has only one sex-determination system that male heterogametic sex determination system (XX/XY) or female heterogametic sex determination system (ZZ/ZW). However, interestingly, Japanese wrinkled frog *Glandirana (G.) rugosa* have dual sex determination system within conspecies, varying with the region where the frog lives. In order to examine where *G. rugosa* frogs with either male or female heterogametic live, we collected frogs from over 180 sites in Japan and South Korea. Using these frogs, we constructed phylogenetic tree based on mitochondrial 12S ribosomal RNA and 16S ribosomal RNA sequences, and analyzed the sex-linked DNA marker. These results uncover that *G. rugosa* frogs are divided into 6 groups (5 groups in Japan and 1 group in South Korea) and we successfully find out new 7th group in Japan that have not reported. We designate these groups East, West, Central, North, West-Central, Southeast Kyushu (Newly found group) for Japanese frogs and SK for South Korean frogs. East, Central and West-Central groups are diverged from Southeast Kyushu group, on the other hand North and West groups are diverged from SK group. And then, North and West-Central groups have female heterogametic sex determination system and the other have male heterogametic sex determination system. In this presentation, I discuss the migration pathway of *G. rugosa* from Eurasia continent to Japan by connecting the phylogenetic tree of *G. rugosa* with the geographic history of Japan, and I demonstrate that androgen and its receptor decide the male sex determination of ZW type *G. rugosa* frog.



### Changes in genetic structure of hybrid zones between two *Gekko* species over 18 years

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Two common nocturnal gecko species, *Gekko hokouensis* and *G. yakuensis* are sympatrically distributed in western Japan. According to previous allozyme analysis conducted in 1999, hybridization between these two species had occurred at four sites out of 18 sites and hybrid swarms were formed at two out of the four sites. To clarify whether the hybridization has proceeded and whether the hybrid swarms have been maintained over 18 years, we investigated their genetic structure using samples collected from 12 sites of the same region in 2017 and 2018. We genotyped 290 individuals using 16 microsatellite loci, and sequenced partial mitochondrial DNA to understand whether the hybridization is reciprocal or not. Results from analyses of their genotypes suggested that 67 individuals were hybrids, either of F1, F2, or backcross with both parental species. Putative hybrids were detected at nine sites. Increased number of sites with hybrids suggests the range expansion of *G. hokouensis* and the spread of hybrid zones. Putative pure *G. hokouensis* dominated at the two sites where hybrid swarms were formed previously. This shift might be caused by exclusion of individuals with hybrid genotypes because of their lower fitness than that of *G. hokouensis*, and/or reduction of encounters between the two parental species due to decrease of pure *G. yakuensis* and invasion of pure *G. hokouensis*. Based on mtDNA analysis, haplotypes of both parental species were observed in F1 hybrids, indicating reciprocal hybridization under natural condition.

**Translocation as a conservation tool for the Bolson tortoise (*Gopherus flavomarginatus*) in northern Mexico**

**Palomo-Ramos R<sup>1</sup>**, Gatica-Colima A<sup>1</sup>

<sup>1</sup>*Universidad Autonoma De Ciudad Juarez*

Translocation programs have largely been used on birds and mammals, however their use on reptiles is increasing. With more than 80% of turtle and tortoise species at risk of becoming extinct in the world, translocation may be an option to recover populations. In Mexico, 33 of the 48 chelonian species, have a protection status but no translocation programs have been conducted. Therefore, we reviewed open literature on worldwide tortoise translocation projects to attempt the development of a guideline to improve success rate. We identified the main influencing factors for a successful outcome: habitat suitability, acclimatization (soft release), selection of translocation candidates, monitoring post translocation and public acceptance of the translocation program and we also propose the Bolson tortoise (*Gopherus flavomarginatus*), a critically endangered species (IUCN), as a candidate for such translocations. The information provided by this review will be useful to develop strategies for future tortoise translocations in Mexico.

### Understanding the Role of the Adaptive Edge for the Desert Tortoise in a Changing Climate

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Understanding how species respond to climate change is important, especially for ectothermic species that thermoregulate. In a changing climate, adaptation is likely to occur at the edge of a species' range, however, many species are primarily sampled in core areas of their range. The Mojave Desert Tortoise (*Gopherus agassizii*) is a United States Fish and Wildlife Service threatened species and is found in the Mojave Desert of the Southwestern US, east and north of the Colorado River. Field surveys have primarily focused on collection of population densities and genetic data in designated critical habitat units for the species, and thus interactions with climate and potential genetic differences at the adaptive front of their distribution have been understudied. These edge areas could potentially become important in maintaining populations of tortoises under future climate scenarios that may result in spatial changes of core habitat. We surveyed edge areas, that are historically under sampled, from 2018 – 2019, and then incorporated locality data into an ensemble habitat model to examine differences between core and edge habitat. The habitat model will help us understand the role edge areas play in maintaining tortoise populations now, and under future climate scenarios, as well as help understand connectivity of these areas to core regions. Future work will include combining genetic data with habitat models, and this combination of information on the adaptive edge will provide us with a multifaceted understanding of how the Desert Tortoise uses a changing landscape across the Mojave Desert.

### **Hacking chemical communication by Burmese pythons in the Everglades**

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The Burmese python (*Python bivittatus*) is a major invasive species of concern in South Florida, especially in the Florida Everglades, because of their broad, negative impacts on native fauna. When and how Burmese pythons locate mates are important questions that, if answered, can improve wildlife management practices. Pythons demonstrate reproductive characteristics common to all snakes that have been studied, specifically mate tracking and courtship by males who are tuned to female chemical signals (pheromones). Previous work established that male snakes of multiple species can be feminized via steroid hormone manipulation. Males implanted with 17  $\beta$ -estradiol become attractive to other males and their skin lipids shift to resembling, if not replicating, those of females. Therefore, steroid manipulation could be used as a tool to improve removal efforts if coupled with existing tools (e.g., Judas males implanted with radiotransmitters). For the past year, we have implanted male Burmese pythons (n=6) with estradiol implants (E2 males) or blank implants (n=6; SHAM) and then tested mate tracking of SHAMs as they attempt to discriminate E2 male trails from those of wild females. We assessed male behavior during the breeding season (December – April) in two ways: using an outdoor Y-maze and in outdoor enclosures equipped with remote sensing technology to track male movement. We predict that E2 males should be chemically indistinguishable from females and that males will spend significant time in the enclosures investigating odor trails from E2 males.

## Landscape genomics of direct-developing frogs along a tropical elevation gradient in Mexico

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Genetic and phenotypic variation is influenced by biotic and abiotic factors, which can be responsible for population divergence and limited gene flow across populations. Abiotic factors, like geography or environmental variables, can limit gene flow and lead to local adaptation. Environmental gradients, such as an elevation gradient, can be an ideal model to study the environmental variables that influence population differentiation and to quantify genetic and phenotypic changes at a local spatial scale. Here, we use a natural occurring elevation gradient of the direct-developing frogs of genus *Craugastor* to examine limits to gene flow and local adaptation. *Craugastor* frogs are widely diverse and their evolutionary history is apparently split by elevation and latitudinal gradients. Our focus is on *Craugastor loki*, a species occurring from 200 to 2100 m. To define the points of sampling, we characterized environmental landscapes of *Craugastor loki* distribution in Chiapas, Mexico using temperature, precipitation and elevation. The sampling was designed to disentangle the geographic and environmental factors influencing population divergence along the landscape. We expect population differentiation to be lower in the same environmental landscapes, and that it would increase as the environmental differences increase. We sampled 12 points of the five environmental landscapes along a 210 to 2130 meters elevation gradient (2 or 3 points per environmental landscape, with a total of 89 individuals). We used RAD-sequencing data to quantify genetic differentiation and landscape genomics analyses were performed. Also, for each sampling frog, we recorded thermal physiological traits.

## Uncovering the function of an enigmatic display: antipredator behaviour in the iconic Australian frillneck lizard

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When faced with a predator, some animals employ a deimatic display to momentarily startle predators, resulting in a pause or retreat, thereby increasing their chance of escape. Frillneck lizards (*Chlamydosaurus kingii*) are characterized by a large, pronounced frill that extends from the base of the head to beyond the neck and when displayed, can be up to six times the width of the head. We used behavioural assays with a model avian predator to demonstrate that their display conforms to deimatic display theory. First, juveniles and adults deployed the frill in encounters with a model predator. Second, the display revealed three colour patches (white and red/orange patches on frill; yellow mouth palate) that facilitate a transition from a cryptic to a conspicuous state as perceived by a raptor visual system. Third, the display was performed with movements that amplified its effect. Frill area was greater for males than females, which suggests that the frill may also be co-opted for male-male contests. If future research confirms the frill's role in male agonistic interactions, frillneck lizards will be a rare case in which a structure has a dual function as both a deimatic display and a sexually-selected signal.

**Morphological study of oriental garden lizard, *Calotes versicolor* Daudin (1802), in Thailand**

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*Calotes versicolor* Daudin (1802) is among one of the most commonly distributed lizards in Thailand. However, based on morphological ambiguity, this nominal species may represent a species complex. Nonetheless, as of present, there is an obvious lack of information regarding the morphological variation. Therefore, this study focused on a detailed morphological assessment of *C. versicolor* in Thailand using 28 mensural characters and 9 meristic characters (n = 182). Furthermore, the study also included a thorough comparison of Thai adult specimens with conspecific specimens from India and *C. irawadi* specimens from Myanmar (Zug et al., 2006 and Pal et al., 2018). In general, specimens from Thailand were relatively smaller, in terms of head width (mean  $\pm$  SE =  $14.72 \pm 0.16$ ), eye-ear length ( $5.35 \pm 0.06$ ), snout-forearm length ( $26.85 \pm 0.25$ ), trunk length ( $38.55 \pm 0.26$ ), and hind foot length ( $23.19 \pm 0.16$ ), when compared to Indian specimens. In addition, with regards to meristic characters, numbers of mid-body scales (38-49) and canthal scales (6-9) of Thai specimens were relatively higher compared to specimens from India. Sexual differences in morphological characters were also observed. Nonetheless, there was no distinct morphological difference between Thai *C. versicolor* and *C. irawadi* from Myanmar. In order to clarify the taxonomic status of *C. versicolor* in Thailand, a genetic assessment should be further investigated.

## Respect thy neighbour: Human, Black mamba and Mozambique spitting cobra interaction in Durban South Africa

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Anthropogenic land-use change especially urbanisation, generally affects biodiversity negatively. However, some species can persist in urban areas. The black mamba (*Dendroaspis polylepis*) and the Mozambique spitting cobra (*Naja mossambica*) are two snake species capable of living in urban landscapes. However, this likely leads to increased human-snake conflict. What is most astounding is although Durban and its surrounding area has a population of 3.4 million people there has been no human fatalities recorded from either of these species post 2015. We are currently investigating aspects of the ecology, behaviour and gene flow patterns of black mambas and spitting cobras in the city of Durban South Africa. Since 2016 we have been retrieving, geographical location, body length and sex of each snake caught, and tail tips obtained for genetic analyses, before their release. To date tail tips have been collected from 123 black mamba and 68 Mozambique spitting cobras from different locations throughout the city. Only three black mamba individuals have been recaptured and no Mozambique spitting cobras. The majority of captures of both species are in urban areas close to remnant natural forest. We plan to examine the specific movement behaviour of individuals using a telemetry method and options are being examined. Experiences and attitudes of the citizens from all sectors of Durban towards these snakes is being recorded. With our continued work on both species in Durban we hope that our research can be transferable internationally to other cities around the world that have populations of potentially dangerous snakes.



## Effects of long-term exposure to snake research on human-snake conflict in Sakaerat Biosphere Reserve, Thailand

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Conservation of reptiles and amphibians often presents unique challenges for outreach and education. Many reptiles and amphibians are less loved, feared and vilified by the public and media, or simply lack the significantly iconic status needed for their viability as a source of tourism and economic prosperity. Outreach programmes for less favourable species call for longer effort and holistic approaches which could lead the way in lasting wider reaching ecosystem conservation.

Snakes are amongst those most maligned animals, with some species capable of inflicting great harm or death they are hated and feared by many across the globe. So significant the denigration of these animals it is even suggested the fear of them is an innate human response felt around the world even in those countries which have few or no venomous snakes.

Here we describe how long-term exposure to snake scientist presence has begun to effect perceptions of researcher and snakes alike within a rural agricultural based community in Northeast Thailand.

Since November 2014 the Naja's Project based in the UNESCO Sakaerat Biosphere Reserve, employed a range of outreach and community engagement initiatives including snake rescue services, snake handling training, education, and production of informative resources. Singularly or in partnership with the Sakaerat Environmental Research Station and fellow scientists. We aimed to increase knowledge of the complex local ecosystems and the place of snakes in them whilst building capacity within local communities. Over time we have observed both positive and negative effects of long-term exposure to field scientists.

**Description of taxonomic and morphological characteristics of tadpole of Murree Hills Frog (*Nanorana vicina*)**

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We described external morphology, morphometry and oral disc structures of the tadpole of Murree Hills Frog (*Nanorana vicina*) for the first time. The species is endemic to Pakistan and India, and bears exceptional conservation value. The tadpoles were extrophic, lotic, benthic, medium sized (43.9-59.94 mm), oval in dorsal view and dorsoventrally flattened in lateral view, nares closer to the eyes than snout, spiracle sinistral, vent tube dextral, tail more than 1.5 the body length with a round tip, anteroventral oral disc, not emarginated, marginal papillae continuous on posterior labium and lateral margins of anterior labium with a wide medial gap, sub-marginal papillae single rowed at the posterior labium and few scattered at the lateral sides of the anterior labium. The keratodont formula was 5(3)/3(1). Scanning electron microscopy revealed that each denticle (stage 36) had 10-13 points, directed inward towards the beak and was club shaped, and the serrations on beak were 11 per 0.5 mm. The tadpoles were categorized as type four larvae of mountain-stream type.

**Notes on natural history of Endemic Murree Hills Frog (*Nanorana vicina*, [Stoliczka 1872])**

**Rais M<sup>1</sup>**, Abbassi S<sup>1</sup>, Gill S<sup>1</sup>, Islam A<sup>2</sup>, Munir S<sup>1</sup>, Razzaq M<sup>1</sup>, Anwar M<sup>1</sup>

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Murree Hills Frog (*Nanorana vicina*) is one of the under studied anuran species. Our data suggest that the distribution range may incorporate areas westward to the range and that the species may not occur in areas south to Rawalpindi such as Jhelum, Gujrat and Sialkot as reported by IUCN. We observed that females ( $67.366 \pm 17.67$  mm) of Murree Hills Frog (*Nanorana vicina*) were larger than males ( $64.390 \pm 7.76$  mm). The single recorded call with a total call duration of 15.4s and with three notes showed 8-9 nodes in each note, 5ms interval between the notes, 3.39 to 4.29 maximum amplitude, -3.03 to -4.61 minimum amplitude and 22050 Hz frequency. The frog species is mainly insectivorous. However, opportunistically it also feeds on other small frogs and vegetation. The food item taxa during breeding and non-breeding seasons did not differ significantly. The similarity index showed 81 % similarity between food item taxa during the two seasons. Few plant species such as *Cyanodon dactylon* and *Trifolium repens* and specimens of insect order Dermaptera were only recorded during breeding seasons while specimens of insect order Homoptera were recorded during non-breeding season. We observed urbanization, deforestation and water withdrawal from the habitat (torrent streams and springs) of the species as major threats to the species. The satellite image of the area surrounding one of the permanent water streams where the species lives and breeds shows high deforestation and urbanization. We have suggested research, conservation and management options.

## Defining cryptic species in the endangered Mountain Dragon: A combined genomics and morphometric approach

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Species with independent evolutionary histories that look morphologically similar hinder taxonomic assignments. When one of these cryptic species is at risk of extinction, incorrect taxonomic assignments therefore affect conservational efforts. Integrative taxonomic analyses including multiple lines of evidence, i.e. genomic and morphological data are needed to overcome this issue. Here we analyze an endangered cryptic species complex of Eastern Australian Dragon lizards (*Rankinia spp.*) to assess the taxonomic status of different *Rankinia* populations and to inform conservation strategies for this group. Preliminary research suggests the presence of at least two different species within *Rankinia*, and one isolated population in the Grampians National Park, Victoria is critically endangered. Using the DArTseq genome reduction technique, we generated a critical dataset of SNPs (single nucleotide polymorphisms) from 86 individuals of different populations of *Rankinia* in Victoria, Tasmania and New South Wales. Furthermore, we applied micro x-ray computed tomography to assess morphological differences of skull shapes of 43 individuals, covering the entire range of *Rankinia*. The phylogeographic structure of our data reflects phylogenies based on mitochondrial DNA, and links the endangered Grampians population to populations on Tasmania / Flinders Island. In addition, our results suggest that populations are genetically divergent, including those within Victoria, which will have important implications for the conservation management of this species.

### **Putting the HER in Herpetology**

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Historically there has been a sex gap in the field of herpetology, with far more male than female herpetologists. Although this has not been formally quantified, the sex gap appears most prominent in research on snakes. We quantified the sex gap in authorship on snake research over the past fifty years, as well as research on lizards, an area which we identified as having a potentially more balanced sex ratio. We hypothesize that the sex gap in authorship has narrowed over the past fifty years in both lizard and snake research, but that a sex gap in authorship still persists, especially in research on snakes. We conducted a literature search from 1970 to 2019 with either the word “snake” or “lizard” as a keyword or in the title. The binary sex (male or female) of authors was assigned to each author based on the sex most associated with the name, and if the sex of the name was ambiguous, we looked up further information on the author to determine sex. We will discuss our findings with respect to author sex as well as other variables including first authorship, times cited, and others.

**Extensive protected area with unknown diversity: the amphibians of the National Park Cordillera Azul, Peru**

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Peru is among the countries with the highest diversity worldwide, representing a megadiverse hotspot for amphibians. With 636 scientifically recognized species, it occupies the third position following the adjacent countries Colombia and Brazil. The National Park Cordillera Azul (PNCAZ), located in a transition zone of Andean and Amazon Basin, represents the largest Peruvian conservation unit occupying an area of ca. 13,532 km<sup>2</sup>. Although PNCAZ has been suggested as a crucial area for conservation, only two preliminary unpublished amphibian inventories were performed in this area, which are briefly discussed herein. Currently, a total of 74 species of amphibians (2 Caudata and 72 Anura), distributed across 10 families and 26 genera are known for the PNCAZ, which corresponds to 11.6% of the total Peruvian amphibian diversity. Craugastoridae (19), Hylidae (12) and Leptodactylidae (11) were the families with the highest species richness. The status of a remarkable part of the sample (28 species) require a more detailed revision, with 18 species of unclear taxonomic status and 10 taxa representing potential candidate species. Regarding reproductive strategies, 21 species exhibit direct development, 20 perform parental care and 45 terrestrial spawning. The considerable number of species that present terrestrial spawning and/or direct development, as well as the expansive number of species with parental care is typical for amphibian communities in tropical moist forests. Our preliminary amphibian species list of the PNCAZ will be useful as the basis for further systematic inventories as part of an ongoing PhD thesis that will provide crucial information for conservation strategies.

**New approach for conservation planning – unmanned aerial vehicles in high resolution predictive population assessments**

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Fast and efficient data collection has long been a concern in applied ecology and land planning. Our study focuses on developing a method for thorough and efficient spatial explicit population assessment of small vertebrate species in on landscape level. We exemplify our approach using *Lacerta agilis* population close to Cologne. In a previous study we have performed species distribution models and population connectivity analyses of *Lacerta agilis* populations based on field surveys and remote sensing data. The resulting maps showing potential habitats and gene flow paths are used by an environmental management agency for conservation planning. In our follow up project, we expand the data sets and develop much more fine scale predictions for habitat suitability and potential population sizes. One major part of this study is the use of very high-resolution remote sensing techniques using a drone, which allow us to quantify not only habitat structure but also microclimatic features. In concert with capture-mark-recapture analyses and radio telemetry, this allows for fine scale assessment of the spatial properties of home ranges and corresponding habitat preference. The study combines conventional field work with novel approaches and has increasing importance nowadays, as detailed small-scale ecological assessments are necessary in land use, construction planning and conservation.

**Allometry and sexual dimorphism in the *Borikenophis portoricensis* (Puerto Rican Racer: Dipsadidae)**

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The *Borikenophis portoricensis* (Puerto Rican Racer) is one of 12 native snake species of Puerto Rico. This colubrid is typically diurnal, oviparous and has a diet predominantly of amphibians and reptiles. Despite being one of the species with the most extensive distribution in the island, very little is known regarding its biology and ecology. Specimens of *B. portoricensis* were captured at Cambalache State Forrest in Arecibo, Puerto Rico. The following variables were recorded for each specimen: snout-to-vent length (SVL), tail length (TL), head length, weight, number of scars, number of ticks (*Amblyomma sp.*) and sex. Linear regression models were used to explore morphometric and allometric relationships among the measured variables between males and females. The results indicate that male *Borikenophis* have a significantly higher body condition index (BCI) than females. Allometric comparison between males and females indicate that males have larger heads per SVL than females. We also found that males have more ticks and scars than females. These results suggest the existence of sexual dimorphism in *Borikenophis portoricensis*. The significantly higher number of scars in males suggest that they are more exposed to predators than females. However, it may also indicate the existence of male-male aggression related to mating. The higher number of ectoparasites observed in males indicates that they are more vulnerable to tick-borne diseases.



### Reproductive data in some oviparous and viviparous non venomous snakes in captivity

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In present times, reptile fauna is facing more environmental problems like never before: Global warming, Deforestation, Industrial and developmental waste, Habitat loss, etc. Snakes are among the most affected groups mainly due to misconceptions and prejudices commonly regarding them as evil or dangerous, killing them on sight, regardless if they are venomous or not. One solution to aid their conservation is captive breeding, in order to support: Population restoration programs, Act as genetic reservoirs and to provide individuals for other research programs. The laboratory of Herpetology at the FES-Iztacala UNAM has one of the largest live collections of reptiles in México, with 19 species of non venomous snakes (150 individuals). In oviparous snakes, eggs were recovered from the enclosure and placed in plastic boxes with (2.5-3cm) depth of vermiculite substrate, and incubated in a bacteriologic stove (RIOSSATM) at (28-30°C) temperature range and with (60-70%) humidity until they hatched. In viviparous snakes, gravid females were placed in separate containers with temperature range (24-26°C) until parturition occurred. From may 2016 to September 2019, thirty-four reproductive events occurred: (17) *Pituophis deppei deppei*, (3) *Trimorphodon biscutatus*, (2) *Lampropeltis triangulum campbelli* and (1) *Rhadinea decorata* hatchlings and (4) *Thamnophis melanogaster* and (7) *Storeria storeroides* offspring. Incubation time and morphometric characteristics were registered for clutches and offspring. The data presented here is from some of the commonly kept species but also includes some of the rare, which might aid to compare and establish captive breeding parameters for them and aid to their conservation.

**Morphology and molecular genetics of the Southeast Asian cascade frogs, *Odorrana livida* complex in Thailand**

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The Southeast Asian cascade frog *Odorrana livida* (Blyth, 1856) has been assumed to be species complex with a wide geographical distribution. In this study, multiple lines of evidence from morphology, morphometrics and molecular analyses (mitochondrial 16S rRNA gene) were used to investigate diversity and distribution in *O. livida* species complex in Thailand. Phylogenetic analysis revealed six well-supported lineages among the five nominal species included in the study. Differentiation among lineages are supported by the great genetic distances between the lineages. Morphometric analysis could not clearly separate between some lineages due to high variation on morphological variables. However, some morphological characters (i.e., dorsum skin, gular pouches, ventral spine and marking on posterior surface of thigh) are useful for species discrimination. Furthermore, these lineages have syntopic distribution in several localities across Thailand. The phylogenetic relationships and their distribution could be determined the conservation status and biogeographic implication of *Odorrana*.

## Endocrine disruption affects physiological ontogeny of a wide-spread anuran

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Environmental change exposes wildlife to a wide array of environmental stressors that arise from both anthropogenic and natural sources. Many environmental stressors with the ability to alter endocrine function are known as endocrine disruptors, which may impair the hypothalamus-pituitary-thyroid axis resulting in physiological consequences to wildlife. In this study, we investigated how the alteration of thyroid hormone (TH) levels due to exposure to the environmentally relevant endocrine disruptor sodium perchlorate (SP; inhibitory) and exogenous L-thyroxin (T4; stimulatory) affects age, size, and the ontogeny of energetics (i.e. resting heart rate (fH), standard metabolic rate, and body condition) before, during, and after metamorphosis in the common frog (*Rana temporaria*). We found that altered TH levels significantly affected growth and energetics at all developmental stages throughout ontogeny. Tadpoles and froglets with high TH levels during development were significantly the youngest, largest and heaviest at all stages compared to Control group and individuals at low TH levels indicating an increased developmental and reduced growth rate. Consequently, effects of altered TH levels are displayed quite fast after first exposure to endocrine disruptors. Whereas SMR was reduced at altered TH levels, high TH levels increased and decreased fH and body condition throughout ontogeny, respectively. Effects of altered TH levels were lowest after completion of metamorphosis. Since endocrine disruption as caused by several environmental stressors may have influence on individual fitness across life since size, age and energetics after completion of metamorphosis determines juvenile survival.

## What role do ephemeral ponds play in the amphibian disease landscape?

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As emerging infectious diseases continue to impact wildlife populations, it is critical to characterize the mechanisms that link habitat, host/pathogen biology, and disease dynamics. *Batrachochytrium dendrobatidis* (*Bd*), has caused amphibian declines worldwide. *Bd*'s aquatic zoospores cannot survive drying. Thus, the multi-year persistence of *Bd* in ephemeral pond communities, is dependent upon infected animals bringing zoospores back to the pond each spring. In contrast, in permanent ponds, overwintering larval and adult amphibians can act as reservoir, allowing *Bd* to persist across years. I predict that hosts that overwinter and breed in permanent ponds but later move to ephemeral ponds will bring new *Bd* strains and increase disease risk. In this case, *Bd* dynamics on the landscape would be coupled between permanent and ephemeral pond communities. Alternatively, *Bd* may be brought to ephemeral ponds by species that emerge infected from terrestrial hibernacula and breed in ephemeral ponds. In this case, the *Bd* dynamics of permanent and ephemeral ponds will be uncoupled.

I am capturing amphibians entering and leaving two ephemeral ponds from the start of breeding until the pond dries by encircling these ponds with drift fences and pitfall traps. I am swabbing the skin of each captured animal and estimating *Bd* infection load. I will use a Fluidigm platform to identify the strain of *Bd* from *Bd*-positive swabs. Understanding how *Bd* spreads to ephemeral ponds and how ephemeral pond communities contribute to the landscape-level dynamics of this host-*Bd* interaction will be important in developing disease mitigation strategies.

**Genetic and conservation of the critically endangered Brazilian snake *Bothrops insularis***

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*Bothrops insularis* is a critically endangered species endemic to Queimada Grande Island, São Paulo state, Brazil. Due to its vulnerability, four Research Institutes keep five populations for breeding and conservation purposes. Genetic approaches of *B. insularis* are still scarce. This study aimed to: (i) characterize and compare the genetic diversity of the ex situ population (housed at the Laboratório Especial de Ecologia e Evolução, Instituto Butantan, São Paulo state) to representatives of the in situ population, (ii) test the presence of genetic structure among and within populations, and (iii) provide data to the conservation program of this species. We used 12 heterospecific microsatellites obtained from three species of the *B. neuwiedi* group to assess genetic diversity indices. Results recovered low to medium genetic diversity parameters for *B. insularis*. The both populations (from the island and the captive one) showed low, although significant values of pedigree (kinship) inbreeding coefficient. Significant values of genetic differentiation indices suggest a little differentiation between populations. Discriminant analysis of principal components (DAPC) recovered four genetic clusters with none geographic relationship, and suggest the occurrence of gene flow among the individuals of the sites sampled. Our data allowed resolving uncertain parental relationship and pointed out to seven preferential breeding to the captive population. A continuous monitoring in both populations is still need, as well as the necessity to bring wild individuals from the island to avoid loss of genetic diversity and inbreeding in the captive population.

**A method for sperm cryconservation of a Mexican small-size lizard (*Sceloporus aeneus*)**

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Currently, efforts to diminish the continuous decrease of reptile biodiversity are insufficient, the inclusion of assisted reproduction techniques could help to improve the conservation of endangered species. Particularly on lizards, few investigations address sperm cryopreservation. Our aim was to develop a non-lethal technique for sperm collection and cryopreservation using egg yolk as diluent and 8% glycerol as cryoprotectant. During mating season, ten males of *Sceloporus aeneus* (were captured on Tlazala of Isidro Fabela, Mexico) were induced to relaxation with pentobarbital sodium (16 mg/kg intracoelomically) to obtain semen by the pressure of genital papilla. Cloaca was cleaned before to prevent contamination. Each ejaculate was diluted up to a final volume of 150 µl. Sperm motility (SM), sperm viability (SV), abnormal morphologies (AM) and membrane integrity (MI) were evaluated (50 µl). Remaining volumes were subjected for cool at a 0.1 °C/min rate up to 5°C while glycerol was adding. Pellets of 40 µl were formed on CO<sub>2</sub> ice (-78°C), before the storage by immersion in liquid nitrogen. Thawing was done by incubation for 3 minutes at 29°C in a 1:1 dilution with the same egg yolk medium. Cryoresistance ratios (Value after thawing/ value before thawing X 100) for each trait were: SM (21.65), SV (30.03), AM (57.56) and MI (31.16). Significant differences were found (T-paired, P< 0.05) between the individual samples of fresh and post-thawed semen. Quantity of glycerol, other diluents and cryoprotectants must be proved to increase motility and sperm survival.

## Differential outcome of chytridiomycosis within species: a review on influential variables

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Since the identification of the infectious agent *Batrachochytrium dendrobatidis* (*Bd*) 20 years ago, numerous studies have helped to build solid documentation on the impact of chytridiomycosis worldwide. *Bd* presence in frogs has been investigated over a broad range of ecological scales. Studies have extended from differences in infection load within individuals, disease prevalence among populations, as well as presence of *Bd* across the landscape level. These studies examined both environmental and intrinsic species-specific factors to explain the variable occurrence of *Bd* within species in order to understand which processes underpin the disease dynamics and differential disease outcome. To evaluate the breadth of studies performed to date we quantitatively reviewed the *Bd* literature, and classified studies with regard to the spatial scale explored, methodological design and overall findings. We systematized which variables were most strongly associated with heterogeneity of disease occurrence. Among the 80 peer-reviewed papers published between 2004 and 2019 that fitted our criteria, air temperature and rainfall or humidity were commonly investigated environmental parameters. Variables such as life stage, distance to urban areas or zooplankton composition were seldom investigated, but were shown to influence *Bd* prevalence or infection load among different populations of the same species. Chytridiomycosis remains a dire threat to amphibians worldwide, and an intricacy of factors influences its occurrence and consequently its effects. Our review emphasises that understanding the complexity of *Bd* impact requires an integration of studies tackling *Bd*-host interaction at multiple scales and perspectives.

## Does Size Matter? Using Osteology and Ancient DNA to Reconstruct Extinct Species Diversity in New Zealand's Large Geckos.

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New Zealand's extant herpetofauna is characterized by high species-level diversity, driven in-part by the repeated formation of allopatric barriers (i.e. mountain-building, glaciations, strait formation and volcanism). However, centuries of anthropogenic disruption (i.e. cryptic extinctions) have obscured true evolutionary patterns, necessitating incorporation of sub-fossil material into existing analyses.

Duvaucel's gecko (*Hoplodactylus duvaucelii*) is a large, monotypic species part of a wider radiation of New Zealand geckos (Diplodactylidae). Sub-fossil evidence (comparably large cranial/post-cranial bones) suggests 'H. duvaucelii' was previously widespread across mainland New Zealand, with subsequent range contraction to predator-free offshore islands following Polynesian/European colonization. This study aimed to reconstruct prehuman diversity in extinct, mainland 'H. duvaucelii' using bone morphology and ancient DNA.

Geometric morphometrics (landmark-based statistical shape analysis) was used to compare 3-dimensional micro-CT scans of sub-fossil gecko maxillae to a reference ontogenetic series (which exhibits species-specific morphometric clustering). Additionally, complete mitochondrial genomes were sequenced from a variety of isolated skeletal elements using state-of-the-art DNA extraction, library preparation and hybridization capture techniques and compared to both published and generated modern datasets. Integrating these aforementioned datasets has enabled robust species delineation of *H. duvaucelii* and identification of fine-scale population structuring, in addition to assigning taxonomic affinity of cryptic species.



## **A metanalysis of snakebites in South Korea: bridging the knowledge gap on snakebite envenomation**

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A thorough understanding of regional snakebite trends is a necessary prerequisite to decrease venomous snakebite. However, the epidemiology of snakebite envenomation is poorly known in many countries, including South Korea. Information on venomous snakebites in South Korea has not been widely disseminated. And it is a significant limitation for understanding regional and global trends of snakebite epidemiology. We have identified published reports on snakebites from South Korea encompassing over 50 years of record. Our metanalysis based on these publications revealed general trends on snakebites in South Korea: 1) men were bitten significantly more than women 2) people in their 50s represented the highest number of bites among age groups 3) most snakebites occurred in agricultural areas and lowland fields 4) most snakebites occurred between May and August 5) fingers and hands were bitten most frequently among victims 6) *Gloydius pitvipers* seem to be responsible for most of the snakebites, with some bite cases attributed to *Rhabdophis lateralis*. Based on our results and on the ecology of Korean venomous snakes, we identified farmers as the population most likely to be affected by snakebites in South Korea. This study is the most thorough analysis of snakebite epidemiology in South Korea to date. However, more work is needed to make detailed risk assessment of venomous snakebites in the country to better mitigate human-snake conflict.

### Natural history of the Central Galapagos snake (*Pseudalsophis dorsalis*)

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The Galapagos snakes (*Pseudalsophis sp.*) are perhaps the least studied terrestrial vertebrate on the Galapagos islands, despite their substantial diversity, with nine species described. There is little knowledge on the Central Galapagos racer (*Pseudalsophis dorsalis*) as the behaviour, colour variation, diet, habitat and population dynamics have not been documented in detail. A standardised method for monitoring its populations and a description of their feeding ecology, will provide a reliable estimate of population health and baseline data for future population comparisons. Analysis of the diet of the snakes will help predict population health by identifying their food requirements and understanding the natural predator-prey dynamic. We performed a population study on Seymour Norte (184 hectares), the northernmost distribution of the Central Galapagos racer. On Seymour there are significant numbers of many Galapagos species including the Central Galapagos racer. We captured and marked 85 individual Central Galapagos racers between 2017 and 2019, subsequently gathering morphometric and colour variation data as well as faecal samples. The Central Galapagos racers on Seymour Norte measure an average of 92.2cm and weigh an average of 97.3 grams. In our sample, we were able to use cloacal probing to accurately determine the sex ratio of 49% males to 51% females captured. Our analysis of diet has so far resulted in Lava lizards (*Microlophus indefatigabilis*) being the predominant prey type in this population, however the identification of other prey items continues. Presence of a marked population may help understand the ecological role of these reptiles in the Galapagos islands.

### The role of the “Tarsia-Crati” Regional Nature Reserve in the protection of the herpetofauna

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The Natural Reserve “Lago di Tarsia e Foce del fiume Crati”, located in the province of Cosenza, has been established by the Calabria Region in 1990. The Reserve is also a Zone of Special Conservation (ZSC) for the Natura 2000 Network, according to the Habitat Directive (92/43/EU).

The Reserve comprises two important wetlands located along the course of the Crati river: the Lake of Tarsia and the Mouth of the Crati river.

The Regional Nature Reserve “Tarsia-Crati” is actively involved in the promotion and management of numerous research projects to protect species of amphibians and reptiles at risk of extinction in Calabria (Southern Italy). In the last years the Reserve has successfully completed a project for the translocation of a population of the endangered tortoise *Testudo hermanni* from a heavily populated area adjacent to the reserve into a natural area within the reserve. Moreover, the Reserve has completed a project for the monitoring of some populations of the European pond turtle *Emys orbicularis* in Calabria. Actually, the Reserve has realized an ex-situ breeding center for the endangered Appenine yellow-bellied toad *Bombina pachypus*, a species that is progressively disappearing throughout its range due to habitat destruction and to the spread of chytridiomycosis. Last spring, the first individuals born in captivity were successfully reintroduced into the wild. The creation of another ex-situ breeding center for the European pond turtle *Emys orbicularis* and for the Italian newt *Lissotriton italicus* is under way and will be completed by the end of spring 2020.

### **Nesting ecology of *Trachemys scripta elegans* in an invaded area in Southern Italy**

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Nesting ecology of slider turtle *Trachemys scripta elegans* was studied in the lake of Angitola, a natural protected area in Calabria (Southern Italy) where the species has been naturalized. From 2008 to 2016, data were collected on 1081 egg shells from 250 nests to obtain information on the biometric and ecological characteristics of the nests. The number of egg shells per nest averaged 4.3 (min-max =1-16) in a clutch with an increasing of clutch size from 2008 to 2010. Two main hatching periods were identified (in July and in September). A significant correlation between nest diameter and nest depth, between nest diameter and egg shell number and between nest depth and egg shell number was found. Regarding the ecological characteristics, we considered the sediment grain size (Te-topsoil, Sa-sand and Ar-clay) and the vegetation cover (meadow, Mediterranean scrub, forest, cultivated areas and mixed areas). The 85% of nests was found in mixed soils (Te-Sa, Te-Ar) and about 14% in homogeneous soils (N = 98, chi-square = 76.351, m.p. = 6, P <0.0001). Regarding the vegetation cover, 54.08% of nests was found in the mixed areas, 34.7% in the meadows, 8.16% in the Mediterranean scrub and 3.06% in forests (N = 98, chi-square = 56.241, m.p. = 4, P <0.0001). An area of mixed vegetation cover reduces the risk of predation on nests and protects them from excessive sun exposure. A mixed and heterogeneous soil allows good ventilation of the nest and a degree of humidity suitable for successful hatchings.

## Investigating the effects of agricultural practices on anuran-trematode interactions

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<sup>1</sup>*Miami University*

Agriculture has the potential to alter host-parasite interactions in aquatic systems by influencing water quality through runoff or drift containing fertilizers and pesticides. Reduced water quality can influence the abundance of parasites and their hosts in ways that increase wildlife and human disease outbreaks. Diseases caused by trematode flatworms, which are increasing globally, are likely influenced by changes associated with agriculture as trematodes have free-swimming stages susceptible to adverse conditions and require snail intermediate hosts dependent on algal food resources. We sought to evaluate how factors associated with agricultural pesticide exposure and changes in snail abundance alter trematode (*Ribeiroia ondatrae* and *Echinostoma spp.*) disease pathology in northern leopard frogs (*Lithobates pipiens*) using a series of mesocosm and field studies. In mesocosms, trematode exposure had negative effects on host development and survival that depended on the presence of pesticides and the abundance of snails. Trematodes caused severe limb deformities, but atrazine, a common herbicide, reduced these effects. Reduced snail abundance in mesocosms, mediated by trematode-induced castration, mitigated the negative effects of trematodes on metamorphs by reducing competition between tadpoles and snails for algal resources. In the field, snail density and parasite prevalence were significantly higher in agricultural ponds when compared to residential or natural ponds, which may lead to increased infections in anurans. These results suggest that anuran-trematode infections may be altered by agriculture in ways that enhance or diminish the effects of parasites on hosts through both direct effects on anurans and indirect effects on algal food resources.

## **Herpetology at the Natural History Museum, London**

**Streicher J**<sup>1</sup>, Cooper N<sup>1</sup>, Campbell P<sup>1</sup>, Gower D<sup>1</sup>, Goswami A<sup>1</sup>, Wilkinson M<sup>1</sup>, Loader S<sup>1</sup>

<sup>1</sup>*The Natural History Museum*

The amphibian and reptile collection of the Natural History Museum, London (NHM) has been maintained for nearly 200 years. Contributors to the collections include key historical figures such as Charles Darwin and Alfred Russel Wallace. The temporal depth of the collection, coupled with dense sampling from the 19th century, offer unique research opportunities - particularly for studying Anthropocene impacts. In this poster we outline current research projects led by staff associated with the section and share exciting collaborative opportunities for herpetologists attending the 9th World Congress of Herpetology. In addition to archival genomics, micro-computed tomography, systematics, species delimitation, macroevolution, and disease ecology research, we discuss other potential uses of large natural history collections (like those at the NHM) that may enhance our understanding and stewardship of the natural world.

## Factors influencing predation risk for tropical lizard eggs

**Swartwout M<sup>1</sup>**

<sup>1</sup>*University Of Arkansas*

For lizard species with high adult mortality, such as small tropical anoles, egg survival is important for population persistence. Ants are frequent predators of tropical anole eggs, but environmental factors can influence their ability to locate or consume eggs. I examined factors influencing lizard egg predation and egg predation risk (based on ant activity at protein-based bait stations) both in a field experiment and in nature at La Selva Biological Station, Costa Rica. I manipulated litter depth and monitored *Anolis humilis* eggs that were placed in litter plots and conducted standardized surveys for lizard eggs in natural nests. In the experiment, egg mortality and predation risk were positively correlated but did not differ across litter depth treatments. The proportion of dead eggs was highest in plots with litter removed, possibly due to higher maximum temperatures and more soil moisture creating less favorable incubation conditions. In surveys, the proportion of eggs encountered that were predated was higher in leaf litter microhabitats and old-growth forest habitat. Additionally, more eggs were encountered in buttresses and fallen logs than open leaf litter microhabitats and egg predation risk was lower in buttresses. Anolis egg survival may be more influenced by whether a nest is in a sheltering object or microhabitat than by leaf litter depth or other local environmental conditions.

## Effect of latitude and urbanization on cold tolerance of an invasive gecko in northern extreme

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Rapid range expansion to high latitudinal areas of an invasive species provides us an excellent opportunity to examine how tropical species respond to the novel environment conditions. However, urbanization would disrupt our prediction by providing extra suitable thermal environment even in a high latitudinal area. The common house gecko (*Hemidactylus frenatus*) showed rapid range expansion at the northern edge of its distribution. We examined the difference in cold tolerance of this gecko along latitudinal and urbanized level gradients near its northern extreme populations. We predict that only the rural populations in high latitude would show lower critical minimum temperature (CT<sub>min</sub>). We used geckos from rural and urban areas from Amamioshima (the most northern extremity of its range) and Okinawajima (little bit south), Japan. The results showed that the individuals from Okinawajima has even lower CT<sub>min</sub> compared to those from Amamioshima ( $F_{2,173}=21.59$ ,  $P < 0.001$ ), being contrary to our prediction. There was no significant difference in CT<sub>min</sub> between the urban and rural populations within Amamioshima, but the former tended to show higher active body temperature in the field than the latter. These results suggest that although the geckos from rural area may suffer more critical thermal environment than those in urban area, the usage of micro habitat may play an important role in the body temperature regulation. The reason for the lower CT<sub>min</sub> in the Okinawajima population is unclear at the moment. We need further investigating habitat usage, ambient thermal conditions, and thermoregulation traits of different types of habitats.



### **Thermal Ecology of an Endangered Lizard**

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The Blunt-nosed Leopard Lizard (*Gambelia sila*) is a federally endangered lizard found in the arid grasslands of the San Joaquin Valley and Carrizo Plain. It has been extirpated from most of its range due to habitat destruction and alteration. Our thermal ecology research examines how climate and landscape features impact lizard thermoregulation, with the ultimate goal of predicting how climate change will impact lizard behavior and habitat use. Specifically, we found that burrows of the Giant Kangaroo Rat provide essential thermal refugia to the lizards. Additionally, Ephedra shrubs allow lizards to be active above ground because they provide valuable shade. We will discuss the results of a study comparing the thermal ecology of lizards in shrubbed and shrubless sites, focusing on the roles of the shrubs and burrows in their thermal ecology.

### **Alpine Lizard Monitoring, Wharepapa/Mt Arthur, Kahurangi National Park, New Zealand**

**Toy S<sup>1</sup>**, Toy R<sup>2</sup>, Hadley L<sup>3</sup>

<sup>1</sup>*Friends of Flora*, <sup>2</sup>*Friends of Flora*, <sup>3</sup>*Friends of Flora*

The community group, Friends of Flora, has initiated monitoring of endemic alpine lizards on Wharepapa/Mt Arthur, Kahurangi National Park, New Zealand. The aim is to document population changes, and understand their drivers, to inform future management. Low impact ink pad tracking tunnels have been used. The footprints of two lizard species, the skink *Oligosoma infrapunctatum* 'Mt Arthur' and a gecko *Woodworthia* 'Mt Arthur', have been identified. Footprints of predatory non-native rodents and mustelids have also been detected. Both lizard species have been found in vegetation-free gaps in *Chionochloa* spp. tussock and alpine cushion vegetation and in more open rocky habitat. Distributions are disjunct, but both species use the same areas. Over three seasons, areas with relatively high numbers of lizard footprints have been identified and will be used in future monitoring to develop indices of abundance. This preliminary monitoring has occurred prior to and during a mass mast seeding of the alpine vegetation. It is anticipated that lizard population trends can be used as an indicator of the health of the wider alpine biodiversity. Use of tracking tunnels is an effective way of involving non-specialist volunteers in alpine biodiversity conservation.

## Space Dynamics and Its Relationship to Arribada of Olive Ridley Turtles at Gahirmatha, India

**Tripathy B<sup>1</sup>**

<sup>1</sup>*Zoological Survey of India*

Sea turtles use beaches that are suitable for the successful development of embryos. Heavy nesting on beaches may have detrimental effects on the survival of embryos as the result of the destruction of nests by con-specific and physiological stress of the incubation substrate. We studied nesting space dynamics and the impacts of nesting Olive Ridley Sea Turtle (*Lepidochelys olivacea*) females on the hatching success in Gahirmatha rookery, along east coast of India. A reduction in beach area following a recent post supercyclone presented an opportunity to test the hypothesis predicting that nest density would negatively affect nest and hatching success. The average calculated area was 475.4 cm<sup>2</sup> for one nest cavity; therefore, the nest capacity of the beach was estimated to be 85,176 clutches. The high degree of nest overlap resulted in relatively low hatching success. We expect, however, that if erosion is arrested, the nesting space is likely to expand and thereby increase long-term hatching success at Gahirmatha along Odisha coast of India.

**Herps in the wind: The community ecology of herpetofauna in windfarms****Trowbridge C<sup>1</sup>**, Withers K<sup>2</sup>, Litzgus J<sup>1</sup><sup>1</sup>*Laurentian University*, <sup>2</sup>*Brookfield Renewable*

Climate change and depleting fossil fuel resources have caused a demand for green energy alternatives, that are low in harmful emissions, but present their own potential problems. Impacts of windfarms on birds and mammals are widely documented, and recent concerns about herpetofauna near windfarms have created a demand for new research. To fill this knowledge gap, in 2016 we conducted a pilot study examining herpetofauna community ecology in a Southern Ontario windfarm situated within an agricultural landscape. We found marginally significantly lower species diversity in wetlands near wind turbines compared to control sites, but post-hoc tests showed that one turbine site was driving the treatment difference. To reduce the influence of other anthropogenic impacts, in May 2018 we began studying herpetofauna community ecology within Prince Wind, a Northern Ontario windfarm located close to relatively pristine wetlands. We are measuring herpetofaunal biodiversity using survey transects, and spatial ecology of turtles using radio telemetry. If herpetofauna are negatively impacted by the presence of turbines, we expect lower diversity in wetlands and turbine avoidance behaviours by turtles. Preliminary data indicate that both treatments have similar diversities with 11 species of herpetofauna observed in both turbine and control sites, and that Painted Turtles (*Chrysemys picta*) in impact and control sites show similar movements patterns. We discuss the implications of our study with respect to conservation of herpetofauna, including possible impacts from windfarms and mitigation strategies. Understanding the interaction of herpetofauna ecology and windfarms is essential to minimizing negative impacts of green energy alternatives.

### Monitoring critically endangered Bermuda skinks

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Monitoring of cryptic or endangered species poses multiple challenges for population assessment and conservation. Failing to account for imperfect detection can lead to biased estimates and misleading inferences about population status. We used a dynamic occupancy model that explicitly accounted for occupancy, detectability, colonisation and local extinction as a tool for assessing populations of the critically endangered Bermuda skink (*Plestiodon longirostris*). The skinks have continued to decline over several decades due to habitat loss and fragmentation, anthropogenic disturbances and predation from numerous introduced species. It is currently estimated that only 1,500 individuals remain across several isolated offshore islands and these could easily be wiped out in the event of a tropical storm or hurricane. Forty sites across Bermuda were monitored between 2015 - 2017 and skinks were found at 13, two of which were new records. Ten observational level and site-specific covariates were combined and considered to influence occupancy and detectability. Our findings provide status assessments that can be used to identify priority areas and interventions for conservation and refine the design and implementation of future surveys for long term monitoring.

### Testes size variation and parental care investment in glassfrogs

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Empirical and theoretical studies have explored how sperm competition, clutch size and variation in hormonal levels affect the relative testis size among frogs. However, male allocation trade-off between fertilization and care behaviors has not previously been considered. Here, we tested whether evolutionary shifts in parental care behavior (presence or absent) are associated with changes in relative testis size. Likewise, we also examined the effects of clutch size, egg size and body male size as alternative factors that might influence testis size in the Centrolenidae family. To test these hypotheses, we collected data on testis size in breeding frogs using information from specimens we have collected, as well as museum specimens, and literature. We measured testes mass, body mass, clutch and egg size from 13 species and six genera of glassfrogs. Controlling for body size and phylogenetic relationships, we found that parental care explained a slight amount of variation in testes mass. Besides, no significant effects of clutch size or egg size were detected. These results suggest that testes size may also vary in response to a post-fertilization investment such as parental care. However, a broader analysis is necessary to corroborate these findings.

## **Behavioural Response of an Endangered Freshwater Turtle (*Clemmys guttata*) to Dynamic Coastal Wetlands**

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<sup>1</sup>Laurentian University, <sup>2</sup>Ontario Ministry of Natural Resources and Forestry

Long-term data are essential to understanding the ecology and behaviour of long-lived species of conservation concern. The natural cycles in lake and coastal wetland water levels can dramatically alter habitat over a turtle's lifespan, so to persist, turtles must show a degree of adaptability to a dynamic environment. We have been studying a Lake Huron, Canada population of globally-endangered Spotted Turtles (*Clemmys guttata*) since 2007. Twelve years ago, during a period of low-lake level, habitat selection of the Spotted Turtle population was studied. That study identified microhabitat preferences and movement patterns that differed seasonally, and found that nesting females showed fidelity to nest substrate rather than location. As high-water levels have returned, we now have the opportunity to compare the habitat use of the same turtles between high water and low water years. Our current project aims to identify whether individual turtles retain these habitat preferences in flood conditions. High-water levels are expected to increase the amount of available shallow-water foraging habitat, and shift nesting habitat inland. If turtles can behaviourally adapt to this natural change in environment, understanding which microhabitat preferences are retained over a range conditions can inform mitigation strategies. Evaluating the potential to construct nesting mounds out of the preferred nesting substrate is one of many possible applications of this research. This research is especially relevant given that human activity tends to suppress natural variation in water levels; variation that is critical in maintaining the early-successional habitat preferred by Spotted Turtles.

### Mapping host distribution using eDNA at the *Bsal* expansion front

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There is increasing evidence that Germany is the European hotspot for *Batrachochytrium salamandrivorans* (*Bsal*). Based on the quantitative monitoring of fire salamander larvae we have identified areas of sudden population collapse, indicating a recent spread of the pathogen. Knowledge of *Bsal*'s expansion dynamics will be essential to predict future spread of the pathogen and to identify areas where mitigation measures need to be taken shortly. However, this requires knowledge of the distribution of potential host populations. For this reason, we developed an eDNA protocol for water samples based on species-specific d-loop primers and probes to map potential caudate host species in the area of expected *Bsal* range expansion. We compared the eDNA results with the result of conventional amphibian mapping from 150 creeks and 150 pools. Control samples were examined from the beginning of the field work to the final laboratory analysis to quantify the rate of false positives. We have not found any false positive records for any of the species. For newts in stagnant waters the rate of false negative results was only between 3 and 6%, while for fire salamanders in creeks (*Salamandra salamandra*) it was comparatively high (29%). As a result, we recommend the use of eDNA for newt mapping in stagnant waters only. Our protocol can also be used to map European newt species in areas where they are themselves invasive (Australia, New Zealand).



**'Lost Generation' – the overlooked scientific value of tadpoles in museum collections**

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Anuran larvae show a wide diversity of morphological features. However, research on frogs and toads is aimed primarily at the adult stage, so that information about their tadpoles is often scarce. In scientific museum collections tadpoles are often barely processed. They remain a 'Lost Generation', and their scientific value is often overlooked. The aim of this ongoing study is to contribute to the identification of phylogenetic and geographical patterns in tadpole morphology using larval samples collected in different regions of the world and stored in museum collections. To achieve these goals, 'classical' museum collection work and modern morphological, molecular, statistical and GIS methodology are combined. The retrieval of available data from museums in combination with available phylogenetic and ecological data will contribute to a better understanding of tadpole morphology.

**Evolutionary Origin and Taxonomic Status of the Freshwater Sea Snake *Hydrophis semperi* Garman, 1881**

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*Hydrophis semperi* is one of only three species of sea snakes that have transitioned from a marine to a freshwater environment. It is found in Taal Lake, Luzon Island, Philippines, which has been isolated from the surrounding marine environment for 265 years. Molecular techniques and linear and geometric morphometrics were used to trace *H. semperi*'s evolutionary history and evaluate its taxonomic status. Twenty-seven cytochrome oxidase I sequences were generated from four species (*H. semperi*, *H. cyanocinctus*, *H. curtus*, *Naja philippinensis*), which were compared along with 15 sequences of three species (*H. brookii*, *H. obscurus*, *Enhydrina schistosa*) from Genbank. Pairwise distance comparisons revealed that *H. semperi* and *H. cyanocinctus* were likely identical. Neighbor joining and maximum parsimony trees of hydrophids based on 565 nucleotides of COI revealed that the two species clustered together monophyletically, with bootstrap supports of 85% and 100%, respectively. Principal component analysis indicated that *H. semperi* and *H. cyanocinctus*' measurements were most proximal to each other relative to the other species analyzed. Canonical variate analysis revealed *H. semperi* and *H. cyanocinctus* shared similar head shape with lowest Mahalanobis distance value (7.4635,  $p < 0.0001$ ). The results from genetic and morphological data suggest that *H. semperi* and *H. cyanocinctus* belong to the same species, with the former being a possible ecomorph of the latter.

### Harlequin frogs in Panama: Is it still possible to find a new species?

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Harlequin frogs genus *Atelopus* (Bufonidae: Atelopinae) occur from Costa Rica southward to Bolivia. About 120 species are currently recognized, however only six of them are known to occur in Panama and the last of them was described from almost 25 years ago. Since then, most of the wild populations of the Central American species were substantially decimated by chytrid fungus, thus one can hardly expect discovery of a new species in the area. During our field research in Eastern Panama we collected several *Atelopus* specimens, which we were unable to assign to any of these known taxa. Further molecular analysis reveals two potentially new species which are now under description.

### What's so Special About the Chaco? (and Why are Chaco Tadpoles so Exceptional?)

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The Chaco is a lowland region in central South America immediately east of the Andes. The Chaco is characterized by distinct wet and dry seasons, and soil rich in impermeable clay. This results in some enormous, yet temporary, ponds during the rainy season. Some Chaco ponds may last up to 11 months, yet the intense dry season assures that they are still temporary ponds and not permanent lakes.

Chaco ponds provide home for aquatic residents, such as anuran larvae, that can metamorphose and leave when the ponds dry up. The ponds' temporary nature limits the type of fish that can live there. Some anuran taxa, most notably in the genera *Lepidobatrachus* and *Ceratophrys* (Ceratophryidae), have evolved to fill niches in the Chaco occupied elsewhere by predacious fish. Many adult features of ceratophryids are associated with fossorial habits and resistance to desiccation during the dry season. This allows them to survive underground when the ponds dry up.

*Lepidobatrachus* and *Ceratophrys* have evolved two very different sets of morphological novelties that make them as both larvae and adults capable of feeding on exceptionally large prey (i.e., megalophagy). Both larval and adult *Lepidobatrachus* can capture large prey underwater. Given their similar feeding ecology, the larvae and adults have as well many morphological similarities. Some unique features of the tadpoles become disproportionately exaggerated in adult *Lepidobatrachus* in a manner unseen in other anurans. *Lepidobatrachus* is unusual in having less morphological differences between its larvae and adults than virtually all other anuran.

### Physical Factors that Influence Tadpole Feeding Efficiency

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Tadpoles of most species feed by grazing upon substrates that may differ in orientation (e.g., vertically along stems, horizontally on the bottom of ponds) and surface texture (e.g., from smooth leaves to rough rocks). We hypothesized that tadpoles' ability to remove food from substrates at different orientations and with different surface textures varies among species depending on the tadpoles' oral morphology. We conducted two experiments to test these hypotheses. In the first we confirmed that the orientation of substrates influenced growth rates for specifically species with different oral morphologies. Whereas some species had high growth rates on specific surface orientations, others were able to grow equally well regardless of the substrate angle. In the second experiment, we found that differences in the number of labial tooth rows and in the marginal papillae row configuration influenced tadpoles' ability to feed on substrates that varied in texture. Tadpoles with a high number of labial tooth rows and few gaps in the marginal papillae row were able to feed on a broad range of substrate textures. Those with either few labial tooth rows or numerous gaps in the marginal papillae row fed better on substrates with specific surface textures.

Collectively our data demonstrated that both the physical properties of substrates and variation in oral morphology influence the grazing ability of tadpoles, resulting in differences in growth rates. The data show how physical features of the environment may influence niche partitioning for tadpoles of species that co-occur in complex aquatic environments.

**A simple viability assay for all life stages of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis***

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The amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, has caused widespread loss of biodiversity, impacting hundreds of amphibian species worldwide. It ranks as the world worst wildlife disease, but mitigation has been challenging as it is an unusual pathogen, requiring the development of new methods to enable experimental research. Despite its pathogenicity, *B.dendrobatidis* is a sensitive organism, and it is often necessary to perform viability estimates to confirm that cells are alive. Existing protocols to determine viability of *B.dendrobatidis* zoospores rely on the intact membranes of live cells excluding the dye. However the other life stage, zoosporangia, have porous walls. We instead assessed staining by methylene blue, which is rendered colourless by enzymes on live cells. We demonstrate that methylene blue (0.01mg/ml for 4 mins) can be used to quantify dead zoosporangia grown as adherent monolayers or in suspension using either microscopy or a colorimetric assay. Methylene blue can also be used to determine zoospore viability, allowing one protocol to be used for all life stages. Accurate quantification of *B.dendrobatidis* survival can aid amphibian conservation efforts by allowing rapid testing for susceptibility to antifungal drugs and other treatments.

**Identification of a novel Sunshinevirus in sidewinders (*Crotalus cerastes*) with pneumonia in North America**

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The Sunviridae are a recently discovered virus family in the order Mononegavirales, which also contains several families of significant pathogens including Paramyxoviridae, Rhabdoviridae, Filoviridae, and Bornaviridae. The only previously known sunvirus is Sunshine Coast virus, found in carpet pythons in Australia with neurologic and respiratory disease. Sidewinders (*Crotalus cerastes*) are rattlesnakes native to the southwestern United States and northeastern Mexico. Two sidewinders in a zoological collection in the southeastern United States died after exhibiting respiratory signs. Histopathology revealed respiratory syncytia and rare intracytoplasmic inclusions as well as lesions consistent with bacterial sepsis. PCR testing was negative for paramyxoviruses and reoviruses but revealed the presence of a novel member of the Sunviridae. This virus had 80% nucleotide identity and 91% predicted amino acid identity with Sunshine Coast virus over a conserved 543bp region of the polymerase gene. A probe-hybridization qPCR assay was designed and found three additional cases, all in sidewinders. Other viperids and colubrids in the same building were negative. Greater copy numbers were seen in tracheal washes than in blood or buffy coats. Next generation sequencing of this virus is underway.

**Isolation and re-connection: the formation of a ring-shaped speciation continuum of *Odorrana margaretae***

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The green odorous frog (*Odorrana margaretae*) around the Sichuan Basin of western China has a ring-shaped divergent pattern. With its micro-ring and replicated contact zones between lineages at various degrees of differentiation, *O.m.* is poised to make an excellent model system for understanding the process of speciation. In this study, we re-constructed its formation. Three populations were initially isolated in glacial refugia at ~1 mya, at the west, the south, and the east respectively. Subsequent postglacial expansion created three contact zones with greatly different patterns and outcomes. Both the south-east and the south-west contact zones had extensive population admixture and produced a gradual transition between the western and eastern populations. Consequently, the southern region has the highest genetic diversity and represents an evolutionary “melting pot.” In contrast, the north-west contact zone, which resembles the overlap zone between two expansion terminals of a ring species, had limited admixture, suggesting a partial reproductive isolation. Further analysis detected several barrier loci and revealed evidence for reinforcement in the north-west contact zone. This case demonstrates a clear process of divergence, which may lead to speciation, and the interaction of multiple driving forces (drift, selection, migration). The initial isolation of the three refugial groups likely plays a central role in the formation of the micro-ring. Even with the presence of considerable gene flow along the ring, the geographical isolation through the arms of the ring and selection at the north-west contact zone appear to drive the expansion of divergence and the evolution of reproductive isolation.



### **Antimicrobial gene expression in alligators**

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Crocodylians have powerful antimicrobial activity in their tissues and blood. This may be the result of a particularly active innate immune system. Proteins involved in the innate immune response are produced in the liver and secreted into the blood serum. To study expression of the relevant genes, we constructed transcriptomes from liver tissue of alligators that had been subject to infection to compare them to liver transcriptomes from uninfected alligators. Expression of innate immune system genes and other antimicrobials will be discussed and compared between infected and uninfected animals.

### **Reviewing the past, present and potential lizard faunae of New Zealand cities**

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Cities and urban processes typically have a negative impact on biodiversity via land cover change, high rates of disturbance and high densities of pest species. Increasingly, however, people are being encouraged and empowered to reduce these impacts through urban restoration and backyard conservation initiatives. Internationally, lizards are a common feature of urban biodiversity, but in New Zealand where many species are threatened, little is known about populations of native skinks and geckos in cities. To explore the potential of cities for the conservation of lizards, we collated knowledge about the current lizard faunae of six New Zealand cities and developed a list of species that would have been present in the locations of these cities prior to human settlement. Comparing the two, we found that, although each of the cities has at least one currently urban-dwelling species, the diversity of lizards in all of the cities has declined dramatically since human colonisation. Patterns of species loss in cities reflect those observed across New Zealand more generally; that is, the loss of large-bodied skinks and geckos, probably resulting from predation by introduced mammalian predators, as well as the loss of regionally endemic species. The high diversity of species that are currently, or were historically, present in the locations of New Zealand cities means that urban restoration involving recovery or reintroduction of populations could have significant benefits for lizard conservation and advocacy.

**Dietary specialization in a fish egg eating specialist, *Emydocephalus ijimae* (Squamata: Elapidae)**

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A sea snake, *Emydocephalus ijimae*, exhibits a unique food habit, invariably eating fish eggs laid on the substrate. This species is considered to require constant food intake, since size of the preys is too small. However, the prey resources available to *E. ijimae* may fluctuate seasonally in their distributional area, subtropical East Asian water. We surveyed seasonal dietary pattern of this species. Field surveys were conducted every month from May 2017 to October 2018 in lagoon of Zamamijima Island, Central Ryukyus, Japan. We checked presence/absence of stomach contents by palpation, and took out them by abdominal massages when existed. The removed fish eggs were sorted, estimated in number, and identified to the species level using mtDNA sequences. Also, we estimated the fish fauna and relative abundance of each fish species in the study sites using eDNA metabarcoding analysis. Stomach contents were obtained from 100 out of 212 captured snakes. The proportion of snakes with stomach contents was high in the summer, but remarkably decreased in the winter. A total of 26 fish species were detected from the stomach contents: 96.2% of total estimated number of nests were of the Pomacentridae and over 75% were of five dominant species. Frequencies of intakes of a few species were significantly deviated from expected frequencies from eDNA, suggesting that some species were preferred or avoided by the snakes. We discuss the possible reasons why *E. ijimae* specializes to several pomacentrid species based on available data for reproductive traits of those species in subtropics.

## **Monitoring of frogs through citizen science in Taiwan**

**Yang Y**

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There are 36 species of frogs in Taiwan. Among them, 15 species are endemic, and 4 are alien. In order to monitor and protect frogs in Taiwan, I have recruited and trained 40-60 volunteers every year since 2003. I encourage volunteers to organize teams to conduct island-wide surveys and upload data to Taiwan Amphibians Database website (<http://tad.froghome.org>). In 2018, there were 64 volunteer teams with more than 600 participants doing surveys at 799 sites, and 238,443 pieces of data were accumulated on the website for the public to look up. According to the distribution hotspots and endangered species locations, 110 Frog Important Areas (IFAs) were selected, which will be monitored every year by volunteers to understand the frog population trends in Taiwan. For monitoring and conservation of amphibians in Taiwan, the Society for Taiwan Amphibian Conservation was established in 2019, and we hope to recruit more volunteers to participate in the monitoring practice.

**Characterizing the composition, metabolism and physiological functions of the fatty liver in *Rana omeimontis* tadpoles****Zhu W<sup>1</sup>**, Zhang M<sup>1</sup>, Zhao T<sup>1</sup>, Jiang J<sup>1</sup><sup>1</sup>*Chengdu Institute Of Biology, Chinese Academy Of Science*

Fat storage is required for the life cycle of many organisms. In most vertebrates, WAT is the primary fat depot. However, in primitive vertebrates (e.g., agnathan group and elasmobranchs), liver is usually responsible for fat storing. Amphibians have unique status in the evolutionary history of vertebrates. Despite it has been recognized that adult frogs use their abdominal white adipose tissue as primary fat depot, how tadpoles store their fat is still inconclusive. *Rana omeimontis* tadpoles use their liver as primary fat depot. In sufficiently fed stage 30-31 tadpoles, hepatosomatic indexes (HSI) might reach up to 7%, and triglyceride accounted for 15% of liver weight. Their liver resembled white adipose tissue in histological morphology. Their liver metabolic composition was unique and characterized by the dominance of maltotriose, arachidonic acid and dipeptides in soluble carbohydrates, free fatty acids and amino acids, respectively. Hepatic fat was the major metabolic fuel of fasted *R. omeimontis* tadpoles, and these animal shared similar reserve mobilization and allocation patterns with mammals. Their hepatic fat is important to fueling late metamorphic climax, and starvation could induce accelerated metamorphic traits in tadpoles with high HSI ( $4.96 \pm 0.21\%$ ) and hepatic fat content, but not in those with similar weight but lower HSI ( $2.71 \pm 0.16\%$ ) and hepatic fat content. To the best of our knowledge, this is the first report that liver can be the primary fat depot in vertebrates with higher evolutionary status than bony fish, and their hepatic fat likely provided body condition signal to modulating metamorphosis.