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frog log

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More Frog Bounties from India's Peninsular Mountains

Chytridiomycosis in Asia

Prevalence and distribution of chytridiomycosis throughout Asia

Figure 14. *Ichthyophis glutinosus* with fresh burn-like wounds. Photo Anslem de Silva

use of small quantities of Paraquat (herbicide) and Dimethione used to control sucking insects and Malathion used to destroy mosquitoes and flies in the Rajarata University Park (Mihinthale, Sri Lanka) may have resulted in mortality in frogs (Chalalochani et al., 2010).

A Final Word

Amphibians in Sri Lanka face a multitude of threats. The rate of depletion of forests and wild life habitats in Sri Lanka is considered one of the highest in South Asia (McNeely et al., 1990). Dwindling marshlands and paddy fields due to rapid urbanization and industrialization have reduced or completely obliterated many amphibian habitats. In addition, many streams and canals are becoming highly polluted or getting dried up. Habitats that are altered for agricultural use are treated with a toxic mix of chemicals with little regard to the impact on wildlife. Never has the need for action to address our issues been greater

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Amphibian Research in Sri Lanka

By Madhava Meegaskumbura, Gayan Bowatte, Kelum Manamendra-Arachchi & Suyama Meegaskumbura

In this article we are highlighting some of the ongoing and recently completed research by the Amphibian Research Group at the Department of Zoology, Faculty of Science, University of Peradeniya, Sri Lanka. Some of the work, especially the taxonomic research by us, is essentially an extension of the rigorous explorations and descriptions carried out by the Wildlife Heritage Trust of Sri Lanka in the late 90s and early 00s.

The Island's inventory of *Pseudophilautus* now stands at 67 species, with a well-resolved phylogeny available for all extant and also nearly 40 undescribed species (Meegaskumbura, PhD Thesis work). While this discovery underlines Sri Lanka's status as a global biodiversity hotspot (Meegaskumbura et al. 2002, Bossuyt et al. 2004), we also show that 19 species of Sri Lankan *Pseudophilautus* have become extinct between 1850 and 1940 (Manamendra-Arachchi and Pethiyagoda 2005, Meegaskumbura et al. 2007), which are known only from their type series in natural history museums, representing an extraordinary ~ 50% of the 38 global amphibian extinctions confirmed by IUCN's Red List. These developments in Sri Lanka are especially pertinent given that they come at a time when amphibian populations worldwide are declining, in many cases inexplicably. Of the surviving Sri Lankan tree frogs, Red List considers a total of 35 species to be threatened with extinction, mandating urgent measures to carryout extensive research based conservation work.

Taxonomy and Systematics

We have so far described 39 *Pseodophilautus* species including several extinct species as being new to science. The extant species descriptions were based on taxonomic explorations carried out

from the mid-nineteen nineties, up to 2005. Extinct species are described from reference collections that were made up to over 100 years ago. Continuing with the taxonomic descriptions, this year, we described two new species, *P. hankeni* (Fig. 1) and *P. schneideri* (Fig. 2) using molecular, morphological and morphometric data (Meegaskumbura and Manamedra-Arachchi 2011). One of these species, *P. hankeni* is a high elevation point endemic species from the Knuckles mountain range. The other species is a mid-elevation species from the South Eastern rainforest region of Sri Lanka, including the Sinharaja World Heritage Site. Both species were named after two eminent biologists and teachers, who have helped enormously in the effort of describing the Sri Lankan frog diversity, Prof. Christopher Schneider (Boston University) and Prof. James Hanken (Harvard University). There are nearly 25 new species of frogs from Sri Lanka which we are in the process of describing.

Fig. 1. (left) *Pseudophilautus hankeni*, a high elevation, point endemic species from the high peaks of Knuckles mountain range, named after Prof. James Hanken for his contributions to teaching and research in Herpetology. Fig. 2. (right) *Pseudophilautus schneideri*, a mid to low-elevation species from the South Eastern rainforests of Sri Lanka, named after Prof. Christopher J. Schneider for his contributions to Sri Lankan amphibian research. Photo: Madhava Meegaskumbura.

We recently highlighted an independent evolutionary lineage that we recognize as a new genus which we named *Taruga* (Meegaskumbura et al 2010). This species is found in montane and submontane regions of Sri Lanka, and both the tadpoles and adults can be distinguished from its sister clade *Polypedates* in terms of several unique morphological features (Fig. 3).

We are also in the process of publishing the results of molecular analyses into evolution of development and breeding strategies of Sri Lankan frogs.

Tadpole Development

We have started studying the development and life history of various tadpole species, including the terrestrial direct developing forms. In Sri Lanka, as currently understood, there is only a single genus, *Pseudophilautus*, which is characterized by direct development. Though a staging table has been made for them, we continue in describing the variation in both tempo and mode within this genus.

Fig. 3. Tadpoles of *Polypedates cruciger* and *Taruga eques;* showing some of the easily distinguishable generic characters such as the absence of a flagellum in *Taruga* when compared to *Polypedates*. Photo: Madhava Meegaskumbura.

We also have put in a lot of effort in documenting the development of amphibians with aquatic development stages (Fig. 4 and Fig. 5). We not only monitor external, buccal and osteological development (all of which are also of value in systematic studies), but also document the environmental and water quality conditions that are needed for their survival. To determine species identity, we grow the metamorphs up to six months (or more in some instances) until they become distinguishable using taxonomic keys (Fig. 6).

Fig. 4. (top) Drawing of *Microhyla rubra* tadpole, showing off the crescent shaped lobe on ventral tail fin, flagellum and anal tube in tail. Photo: Gayan Bowatte. Fig. 5. (lbottom) A full body photo of *Ramanella obscura*. Photo: Madhava Meegaskumbura.

Fig. 6. Newly emerged froglets of *Taruga eques*. Photo: Madhava Meegaskumbura.

We are also looking at how the habitat and environmental parameters affect the body shape of tadpoles and adults. Using *Polypedates cruciger*, we have found that predator presence affects the body shape of tadpoles and also that prevalence of predators induces early metamorphosis. These results will be published soon (Fig. 7).

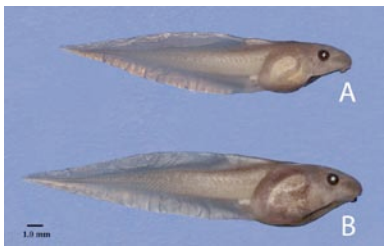

Fig. 7. Differences in body shape in tadpoles reared A. in the presence of a predator and B. in the absence of a predator. Photo: Madhava Meegaskumbura.

Diseases and emerging threats

From the vicinity of University of Peradeniya, we are noticing a group of heavily malformed tadpoles of *Polypedates cruciger*. Observations on development of these tadpoles have shown that the malformations at base of tail region carry through even to juveniles after metamorphosis. We are now studying how these malformations are affecting jumping performance of these frogs, as they become adults and also if malformations affect also the reproductive capability of these frogs. Detailed studies on this

system will reveal not only the causes of malformations, but also how these malformations affect these frogs if they are to survive to reproductive age (Fig. 8, 9, 10).

Over the past ten months we have begun noticing greater incidence of fly attacks on the foam nests of *P. cruciger*. So far, we have observed nearly 15 foam nests of these frogs, of which five were infected with fly larvae. These larvae often destroy entire nests or sometimes tadpole survival is dramatically reduced. There seems to be a correlation with the advent of rain and fly infections; we continue in gathering more data to test this

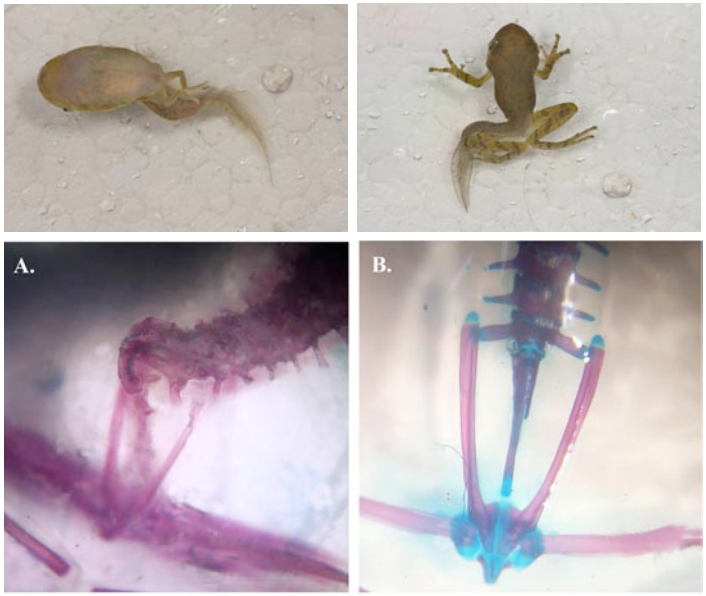

Fig. 8. (top left) A severely malformed *P. cruciger* tadpole swimming upside down, Fig. 9. (top right) Malformed froglet of *P. cruciger* ready to metamorphose. Photo: Madhava Meegaskumbura. Fig. 10. (bottom) A. Deformed urostyle region of a double stained froglet and B. the normal condition. Photo: Gayan Bowatte.

hypothesis statistically. Surprisingly, we are not noticing fly larvae attacks in nearly 15 *Taruga equ*es nests that we observed; however we are not sure if this is due to the absence of the fly species under issue in *T. eq*ues habitat or if these nests are tolerant of these fly attacks.

We also continue to swab the amphibians to determine the presence of the dreaded Chytrid fungus, which if spread in Sri Lanka could potentially cause havoc as has been observed in many regions of the world. Studies done on Sri Lankan frogs so far indicate that the fungus is probably present in Sri Lankan frog populations, mandating urgent continuation of monitoring activities.

Amphibian breeding observations

We are now observing the breeding patterns of various frog species (Fig. 11). We are concentrating on the endemic forms in the wild, and later when we get government permission, we are hoping to also do captive breeding trials, not for reintroduction, but to fine tune

Fig. 12. Measuring microhabitat parameters in the field. Photo: Madhava Meegaskumbura.

Fig. 11. *Pseudophilautus femoralis* is one of a very few species that lay direct developing eggs on leaves. Photo: Madhava Meegaskumbura.

the conservation effort, by understanding the critical conditions needed for their breeding and factors needed for survival of various life history stages.

Function of foam nest material

We are studying how foam-nesting material may facilitate the survival of eggs and early stage tadpoles within the foamy mass in both Polypedates and Taruga. We are now beginning to test chemical composition and protein structure of the material that the foam nests are made with. We are finding that the blue color observed in *P. cruciger* nests is due to Cu^{2+} ions. We are also testing the anti-fungal and anti-bacterial nature of the foam nest material.

Amphibian microhabitat monitoring

Shrub frogs are restricted largely to the rain-forested southwestern 'wet-zone' region of the island, where annual precipitation usually exceeds 2000 mm (only a single species is restricted to the dry zone). Many of these frogs are specialized in habitat use (Meegaskumbura and Manamedra-Arachchi 2005; Meegaskumbura, PhD thesis work). Of the 43 extant species, it is of concern that as many as 15 are known only from a single site each, and 11 from only two—usually nearby—sites each (Manamendra-Arachchi and Pethiyagoda 2005; Meegaskumbura and Manamendra-Arachchi 2005). Since 1815, about 95% of Sri Lanka's rainforests have been lost to coffee, cinchona and tea plantations (Meyer 1998), supporting the idea that extinctions are mainly caused by habitat loss.

We also suspect that recent climatic changes may also have served to stress the shrub-frog populations. Bahir et al. (2005) show for several highland species, that breeding only occurs during periods of sustained rainfall that is reflected by continuously high relative humidity (80-100 %). While historical relative-humidity data are lacking for Sri Lanka, trends calculated by Schaefer (1998) show that at Nuwara Eliya (1,800 m elevation) in central mountains, average annual temperature had increased by 1.3° C and average annual precipitation had decreased by ~20 % in the period 1869-1995; similar desiccation and warming trends were noted also in other parts of the country.

To determine causes of threat by understanding not only the major features of their habitats, but also the more subtle, yet equally important, microhabitat utilization patterns and changes in microclimatic conditions that are important for their spatial distribution, reproduction and survival. With this in mind, we are now starting to monitor the habitats and populations of various frog species in several bioclimatic zones within Sri Lanka, including the Knuckles WHS (Fig. 12), Morningside and Center for Tropical Forest Science's 25 ha plot at Sinharaja.

We are also in the process of starting a habitat enrichment project for threatened species.

Environmental toxicology

Most of the amphibian breeding habitats are polluted by nitrates, the main source of nitrate to the fresh water bodies in Sri Lanka are through excessive usage of nitrate containing fertilizers. The effects of nitrates on the development of amphibians are poorly known for Sri Lanka. We are now documenting naturally occurring levels of nitrates in amphibian breeding sites, and the species inhabiting those, in various bioclimatic regions of Sri Lanka throughout a year.

Experimentally, tadpoles of four species are exposed to predetermined nitrate concentration series and changes in development (developmental anomalies) are being observed. The levels of nitrates in most water bodies fluctuate dramatically, depending on factors such as amount of nitrate fertilizers used, bio-load, time of day, rainfall, drought conditions, distance from cultivated areas to amphibian habitats etc.. Because of this it is extremely difficult to test for the effects of nitrate on amphibian development in the field. Hence controlled laboratory experiments are essential to carry out this research. The conclusions of this research will be important for amphibian species conservation in Sri Lanka.

Bioacoustics

We are using bioacoustics to distinguish between sister species, and we have already used vocalization data even in describing species. However, so far, we have not used bioacoustics to differentiate and monitor frog populations. Now we are documenting all frog calls of Sri Lankan species, so that frog populations can be monitored remotely with least disturbance to the species.

Restoration ecology

We are currently carrying out the habitat monitoring work with the intention of restoring habitats of several critically endangered species of amphibians. We will be providing more details of this as these studies progress.

Paleontology

We have started work on an ambitious project to document the paleodiversity and the paleoenvironment of the known fossil beds in Sri Lanka (Fig. 13). However, we are yet to find any frog remnants from these fossil beds. Two students are currently going through various collections to determine the paleodiversity of these beds.

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The Department of Wildlife Conservation of Sri Lanka and the Forest Department of Sri Lanka have been quite supportive in granting permission to carryout this work; when the restoration component of the work begins, we are hoping to actively collaborate with these two organizations and also other organizations that work for conservation of our amphibians and their habitats. We wish to acknowledge the Amphibian Specialist Group (IUCN/SSC), Global Wildlife Conservation, Amphibian Redlisting Authority (ARLA/IUCN/SSC), Center for Tropical Forest Sciences (CTFS) for support in various forms. Rohan Pethiyagoda, Christopher J. Schneider, James Hanken, Simon Stuart, Don Church, James Lewis and Ariadne Angulo are acknowledged for their support and contributions to Sri Lanka's amphibian research/conservation efforts.

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Fig. 13. Searching for Miocene fossils in the Aruwakkalu fossil bed, N.W. Sri Lanka. Photo: Madhava Meegaskumbura.

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