The conservation breeding of two foot-flagging frog species from Borneo, *Staurois parvus* and *Staurois guttatus*

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Abstract.—The Bornean frogs of the genus *Staurois* live exclusively along fast-flowing, clear water rainforest streams, and are famous for displaying a variety of visual signals, including foot flagging. Their extraordinary behavior, and the continued loss of their natural habitat due to deforestation and subsequent pollution, make them a group of target species for captive breeding, as well as behavioral research. The Vienna Zoo has pioneered in the development of a research and conservation project for *S. parvus* and *S. guttatus*. We implemented two breeding and research arenas, offering an artificial waterfall and different options for egg deposition in a bio-secure container facility. Two months after introducing the frogs, we observed amplexant pairs and the first tadpoles of *S. parvus* and *S. guttatus*. The Vienna Zoo is the first zoo worldwide that has succeeded in breeding foot-flagging frog species and meanwhile has recorded over 900 tadpoles and at least 470 juveniles. One of the most striking observations has been the use of foot-flagging signals in recently metamorphosed *S. parvus*. This corroborates our assumption that “foot flagging” is employed as an intraspecific spacing mechanism. The breeding success of two *Staurois* species at the Vienna Zoo can help in species conservation as it increases our knowledge on conditions necessary to breed tropical stream-dwelling anuran species found to be particularly threatened in nature. Furthermore, the captive colony provides research conditions to better understand the role of “foot flagging” as a visual signal component in anuran communication.

Key words. Amphibia, anura, bio-secure management, conservation research, *ex situ* breeding


Introduction

Amphibian species are declining in many parts of the world. On average 41% of amphibians are classified as Threatened on the International Union of Conservation of Nature (IUCN) Red List. The extinction risk in South East Asia still increases (Hoffmann et al. 2010). Only recently an Amphibian Conservation Action Plan has been developed, which states important priorities for relevant amphibian research and conservation. Understanding the cause of decline, assessing changing diversity and implementing long-term conservation programs are some of the immediate interventions necessary to conserve amphibians (Gascon et al. 2007). Zoo-based amphibian research and conservation breeding programs facilitating *ex situ* and *in situ* conservation of amphibian species have been established for a wide range of species over the last decades (Browne et al. 2011; Gagliardo et al. 2008; Lee et al. 2006; McFadden et al. 2008).

In South East Asia, habitat loss and destruction is one of the main causes for the rapid decline of amphibian species (Stuart et al. 2004). Deforestation of natural habitats increases siltation and chemical pollution in streams. Few stream-dwelling Bornean species are able to survive in habitats modified for human use (Inger and Stuebing 2005). A recent study carried out in Brunei demonstrated that deforestation due to road construction enabled *Limnonectes ingeri* to migrate more than 500 m into primary forest, which posed a potential threat to native amphibian assemblages (Konopik 2010). Inger and Stuebing (2005) mentioned an increase of the Giant river frog (*Limnonectes leporinus*) along silted streams of logged areas and a simultaneous decrease in some species of Torrent frogs (*Meristogenys* spp.). About half the frog species in Southeast Asia are restricted to riparian habitats and develop in streams (Inger 1969; Zimmerman and Simberloff 1996). Most anuran stream-side communities in Borneo are known to breed in clear, turbulent water and are absent in streams with silt bottoms that are lacking riffles and torrents (Inger and Voris 1993). The heterogeneity of riparian habitats in pristine rainforests results in reoccurring stream assemblages and habitat specific adaptations (Keller et al. 2009).
Many stream living anuran species in Borneo show morphological and behavioral adaptations to torrential streams and waterfalls. For example, the tadpoles of *Huia cavitympanum* and of all species of the genus *Meristogenys* have large abdominal suckers specialized for a life in currents (Haas and Das 2012). The adult males of *M. orphnocnemis* use high frequency calls to communicate in noisy stream environments (Boeckle et al. 2009; Preininger et al. 2007). An extraordinary spectral adaptation to enhance the signal-to-noise ratio has also been reported in *Huia cavitympanum*, in which males call in a band of ultrasonic frequencies (Arch et al. 2008). In the vicinity of waterfalls and fast-flowing streams, species of the genus *Staurois* display an exceptional behavior termed, “foot-flagging” (Grafe et al. 2012; Grafe and Wanger 2007; Preininger et al. 2009). The conspicuous visual display mainly observed in tropical anuran species inhabiting riparian habitats (reviewed in Hödl and Amézquita 2001) may act as a complementary mode of communication in noisy habitats.

The Bornean foot-flagging species, *Staurois guttatus* (Fig. 1) and *S. parvus* (Fig. 2) occur in sympatry, but use different microhabitats along streams. Both species have solved the problem of continuous broadband low-frequency noise by modifying their advertisement calls to increase in pitch and use numerous visual signals (Grafe et al. 2012; Grafe and Wanger 2007). Males of *S. guttatus* perch on vegetation along fast flowing streams and waterfalls. Individuals of *S. parvus* display along steep rock formations close to the waterline (D. Preininger, pers. observ.). The breeding behavior and habitat of tadpoles are unknown from *S. parvus*, though given the microhabitats of the adults tadpoles probably live in currents along the stream. *Staurois guttatus* tadpoles, however, have been found in leaf litter in side pools of streams (Haas and Das 2012) similar to an unidentified Bornean tadpole of a ranid genus with slender body shape and nearly pigment-less skin resembling neotropical centrolenid larvae (Ingber and Wassersug 1990). *Staurois parvus* has recently been resurrected from the synonym with *S. tuberilinguis* (Arifin et al. 2011; Matsui et al. 2007). The tadpoles of *S. tuberilinguis*, reported by Malkmus et al. (1999), exhibit a fossorial life in leaf litter at the margins of forest streams. The IUCN Red List categorizes *S. tuberilinguis* as “Near Threatened” with a decreasing population trend (Inger et al. 2004), and *S. parvus* and *S. guttatus* are listed as “Data Deficient” (IUCN 2011).

In 2008, in light of the “Year of the Frog” campaign initiated by the World Association of Zoos and Aquariums (WAZA) and the IUCN we started a unique conservation and research project. A bio-secure container facility was constructed and with permission of the Universiti of Brunei Darussalam and the Brunei Museums Department we imported ten individuals of *S. guttatus* and ten individuals of *S. parvus* to the Vienna Zoo. Apart from several research aspects concerning the remarkable multimodal (visual and acoustic) signals employed in communication, we were especially interested in the reproductive behavior and the accompanying conditions crucial for reproductive success. We here report our first findings in *ex situ* management and breeding of *S. parvus* and *S. guttatus*.

![Figure 1. Male and female *Staurois guttatus* in amplexus resting at a waterfall. Image by M. Böckle.](image-url)
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**Methods**

**Study species**

In May 2010 we collected 20 individuals (ten pairs) of the species *S. parvus* and *S. guttatus* in the Ulu Temburong National Park, Brunei Darussalam, Borneo. Frogs were located at narrow, rocky (black shale) sections of the Sg. Anak Apan and Sg. Mata Ikan (Fig. 3), two small freshwater streams that merge into the Belalong River close to the Kuala Belalong Field Studies Centre (115°09´E, 4°33´N). *Staurois parvus* is a ranid frog, endemic to Borneo. Males are diurnal and perch on rocks along fast-flowing forest streams. Their white chest and webbing between the toes of the hind legs strongly contrast to their cryptic dark grey, brown dorsal body. The snout-urostyle length and weight of the investigated population of male *S. parvus* averaged 21.5 ± 0.5 mm (*n* = 13) and 0.7 ± 0.05 g (*n* = 13) (Grafe et al. 2012) and of females 29.5 ± 1.8 mm (*n* = 5) and 1.7 ± 0.2 g (*n* = 5) (Preininger et al., data not shown). The closely related species *S. guttatus* occurs throughout Borneo. It was previously known as *Staurois natator* (Inger and Tan 1996), a name still used for populations in the Philippines (Iskandar and Colijn 2000). Males of this diurnal species perch on rocks and branches along fast-flowing mountain streams. Females were found 10-50 m away from the river under overhanging rock formations and tree branches. The snout-urostyle length and weight ± SE of the investigated population of male *S. guttatus* averaged 33.6 ± 0.4 mm (*n* = 14) and 2.69 ± 0.07 g (*n* = 14), that of females 50.1 ± 0.3 mm (*n* = 6) and 9.74 ± 0.2 g (*n* = 6) (Preininger et al., data not shown).

Individuals were collected with permission of the Brunei Museums Department.

**Ex situ breeding facility**

In the Vienna Zoo two connected bio-secure containers, fully isolated from other facilities were implemented as the research complex for the animals (Fig. 4). The use of converted shipping containers for the *ex situ* breeding and management of amphibians was pioneered by Gerry Marantelli at the Amphibian Research Centre (ARC) in Melbourne, Australia. The Vienna Zoo has tested specimens (including *S. parvus* and *S. guttatus*) for infection with the chytrid fungus and no positives were detected. At the start of the project we kept individuals in pairs in medium sized terraria (50 × 60 × 70 cm) in the container facility that contained some tree branches, plants, stones, and flowing water which ran over potsherd. We also built a research arena (150 × 120 × 100 cm) for behavioral experiments that we converted into a breeding arena in 2011 (Fig. 5) to improve space requirements because neither of the species had reproduced in their original terraria. We implemented a controllable waterfall with several smaller cascades creating areas of flowing and dripping water that additionally increased humidity levels. Small burrows, ledges, and perching sites were built out of foamed polystyrene. Similar to the smaller terrariums we added plants with large leaves (*Monstera* sp., *Philodendron* sp., *Spathiphyllum* sp., *Dieffenbachia* sp., *Figure 2.* A male of *Staurois parvus* displaying the white interdigital webbing during foot-flagging behavior. The visual signals are mainly employed during male-male agonistic interactions. Image by D. Preininger.
Aglaonema sp., Scindapsus sp., and others) as nightly resting sites. We incorporated a self-built rain and misting system to simulate rainy and dry periods. The water area, which covered the entire floor of the terrarium, was filled with gravel of different grain sizes and larger pebbles that provided perching sites and interstitial spaces. We further installed two smaller glass containers (30 × 30 × 30 cm), one placed directly under the waterfall mimicking a constantly flushed pool with large stones, and the other containing sand, dead leaves, and standing water, as found in side ponds of waterfalls. A mixture of osmosis-purified water and drinking water (average conductivity = 9 µS/cm, pH = 7.2) was discharged via the waterfall and drained into an external filter reservoir, which created a slow current in the main water area. As light source we used a metal-halide lamp (HIT-DE 70 Watt [Daylight]) and placed several plastic boards on top of the terrarium to mimic canopy coverage. Individuals were housed under 12-hour light, 12-hour dark cycles. We placed five pairs of *S. parvus* into the arena. From then on individuals could only be counted at night when perching on leaves, while frogs rested in the many hiding places during the day.

A similar facility (150 × 150 × 150 cm) was constructed for *S. guttatus*, however the water area did not contain additional artificial pools or ponds, and the waterfall was amended with several tree branches. Temperature in both facilities averaged 25 °C (range: 22-27 °C) and closely resembled the natural habitat temperature (Fig. 6). Relative humidity ranged from 95% to 100%. For a period of 14 days we simulated a dry period with no rain and decreased water levels (10 cm), followed by a 14 day rainy period with four hours daily rainfall (7-8am and 5-8pm), elevated water levels (15 cm) and an increased quantity of water flowing over the waterfall. This procedure was repeated with the intervals between the dry and rainy periods reduced to seven days, and rain periods adjusted to different times of day (e.g., 5-10pm and no morning rain). We also played back conspecific advertisement calls recorded in the field, during peak activity periods (9-11am and 3-5pm).

Adult frogs were fed with gut-loaded House crickets (*Acheta domesticus*), Firebrat (*Thermobia domestica*), and blow flies (*Lucilia* sp.); tadpoles received algae tablets, fish food flakes, and fish filet; the diet of metamorphosed frogs consisted of *Drosophila* sp. and *Collemboidea*. All feeder insects were dusted with a vitamin and mineral mixture (Vitakalk, Korvimin or Nekton MSA).

Tadpoles were photographed in petri-dishes on graph paper and snout-vent length (SVL) and Gosner stage (Gosner 1960) derived from the photos. We measured SVL and body mass of juvenile *S. parvus* with a sliding caliper to the nearest 0.1 mm, and a digital mini scale to the nearest 0.01 g. Tadpole specimens of various stages of *S. parvus* were deposited at the Austrian Natural History Museum (*Staurois parvus* larvae: NHMW 39337).
Results

Staurois parvus

On 18 October 2011 we observed the first three tadpoles of *S. parvus* during an evening census of adult individuals in the gravel of the slow-flowing current area of the terrarium. When a tadpole could first be captured it was in Gosner stage 25 and measured 11.2 mm in total length (SVL: 3.3 mm, *n* = 1) and was completely transparent (Fig. 7). Due to the transparency of the body, the organs and blood vessels shined through the skin and the body was of reddish appearance. The highly photophobe individuals colonized the interstitial spaces of the gravel area. More tadpoles staged 26-28, captured 24 days later, measured ca. 21 mm in total lengths (SVL: 6 mm, *n* = 1) and the body and tail were covered with dorsal black spots. After complete toe development (> stage 38) individuals showed a brown coloration with green iridescence and a yellow iris, as seen in adults. At this stage, 70 days after the first sighting, individual length was 41 mm (SVL: 12 mm, *n* = 1). At the end of metamorphosis the dorsal coloration of individuals turned into bright green (Fig. 8).

The first metamorphosed *S. parvus* left the water on 30 January 2012 (SVL: 13 mm, tail-length: 6 mm), 104 days after we observed the first tadpoles. To date, we house 285 froglets in separate terraria in the bio-secure container, over 600 tadpoles and 180 juveniles have been raised for approximately 30 days and afterwards released at an artificial waterfall in the Rainforest house of the zoo (Fig. 9), where the establishment of a semi-wild population is intended. The metamorphs have dark green or black spots and small tuberculi on the dorsal side, the latter eponymous for the closely related species *S. tuberilinguis*. They measured 11.8 mm (mean SVL, SD ± 0.8, *n* = 20) and had a body mass of 0.12 g (SD ± 0.03, *n* = 20).

Due to the high reproductive success we recently allowed disturbance at the setup in order to search for egg-deposition sites. So far, we have discovered two clutches of eggs that were attached under big stones in the slow-flowing water current. Surprisingly, with respect to the large tadpole numbers in the project, those two clutches contained only 14 and 26 eggs, respectively. The survival rate of 120 separated tadpoles (tank A: *n* = 40, tank B: *n* = 80) was 87% (tank A: *n* = 34, 85%; tank B: *n* = 71, 88.8%). Presently, we house over 200 tadpoles, 6-10 juveniles and nine adults in the breeding facility.

Metamorphosed frogs were placed into separate terraria, only hours after leaving the water, and were immediately observed to display foot-flagging behavior (Fig. 10). The young frogs performed complete foot-flags, in which the leg is raised and the toes are spread as observed in adult individuals. Interdigital webbings were colored transparent grey and did not exhibit the contrasting white coloration as seen in adults.

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amphibians have gained global support and resulted in increased conservation efforts for many threatened species (Browne et al. 2011). Information on natural history, reproduction modes, and behavior of anurans is important to determine and protect key-habitats.

The tadpoles of *S. guttatus* and *S. parvus* colonized the hyporheic interstitial in the slow-flowing current areas in the breeding facility, which supports our assumption that the larvae develop in fresh water streams or adjacent pools of fast-flowing mountain streams and waterfalls. On two occasions we found eggs of *S. parvus* in under-water gaps between larger rocks and the subjacent gravel of our breeding terrarium. Neither in the artificially flushed pool with large pebbles, nor in the sand and leaf filled container mimicking a side pool of the waterfall, tadpoles or eggs could be observed. In a stream-dwelling, foot-flagging species from Brasil (*Hylodes dactylocinus*) males dig underwater chambers prior to courtship and eggs are deposited on the sandy bottom between rocks along streams (Narvaez and Rodrigues 2005). Another diurnal species (Micrixalidae: *Micrixalus saxicola*) displays foot-flagging signals and lives along perennial streams in the Western Ghats, India. Females of *M. saxicola* dig under-water cavities with the hind legs in gravel areas of flowing streams while in amplexus with a male or before courtship (Gururaja 2010; D. Preininger, pers. observ.). Although we did not observe *S. parvus* males or females digging under-water chambers, we assume that sufficient gaps between rocks could provide similar protection from predators. We observed amplexant pairs at the study site in Brunei to repeatedly move up the stream only to dive back into pools at the bottom of cascades and smaller waterfalls over a period of 1-2 days. This behavior could indicate either the search for suitable deposition sites or the deposition of several clutches.

**Discussion**

The combined efforts of members of the Vienna Zoo, University of Vienna, and the Universiti of Brunei Darussalam have established a research and conservation project that succeeded to breed the foot-flagging frogs *Staurois guttatus* and *S. parvus* *ex situ*. Zoo-based research and conservation breeding programs focusing on
The diversely structured artificial habitat in the breeding tank offered individuals similar conditions as observed in the natural habitat. Earlier studies that kept adults of *S. parvus* in terrariums of simpler design (no flowing water) showed that individuals did not display acoustic or visual signals under such conditions (R. Kasah, pers. comm.). At the beginning of our project we kept individuals pair-wise in simpler terraria with a small water area containing no gravel and only larger pebbles, some tree branches, flowing water via a pump, and temperatures of 23-25 °C. Under these conditions individuals performed advertisement calls and foot-flagging behavior but no reproductive behavior could be observed. Especially in *S. guttatus* females displayed territorial calls and foot flags if males approached, a behavior that was interpreted as a spacing mechanism (Preininger et al., data not shown). After transferring all individuals in the considerably larger and diversely structured breeding tank, calling activity intensified, and pairs in amplexus could be observed after a few weeks. Hence, we suggest that first and foremost the gravel containing flowing water area was crucial for reproduction, but also the simulated dry and rainy season might have had an effect. It is now essential to alter or exclude single environmental conditions or habitat structures to determine factors necessary for reproduction. So far we have removed the artificial side pool and flushed

**Figure 7.** Tadpoles of *Staurois parvus*. Image by N. Potensky.

**Figure 8.** Juvenile *Staurois parvus*. Image by D. Zupanc.
pool from the *S. parvus* breeding terrarium and still observe freshly hatched tadpoles.

Freshwater streams and adjacent flown-through pools with gravel areas seem to be important to secure the survival of the foot-flagging species in the genus *Staurois*. However, deforestation and subsequent siltation of streams and rivers are the major threats to most stream-living and breeding anuran species in Borneo. Inger and Voris (1993) found that a stream with a silt bottom completely lacked all the species known to breed along clear and fast-flowing streams. Selective logging changes the water chemistry considerably in nearby streams and sediment yields of streams are 18 times higher for up to five months after logging (Douglas et al. 1993; Douglas et al. 1992). So far, it is not well-understood how habitat loss or alternations will affects riparian anurans on Borneo, but considering the dramatic decline of this group of vertebrates it is expected that biodiversity will decline considerably if ecosystems continue to degrade.

For some species *ex situ* programs may be the only option to avoid extinction (e.g., the Kihansi spray toad, *Nectophrynoides asperginis* [Krajick 2006] or the Panamanian golden frog, *Atelopus zeteki* [Zippel 2002]). Species that are not considered Critically Endangered should be preserved in the wild through protection of key habitats and monitoring. Nevertheless, to identify habitats necessary for survival of a species and subsequent immediate protection requires extensive research and conservations efforts. Captive breeding programs however should be extremely cautious to avoid disease transmission, hence in our project only individuals from the bio-secure container facility will be considered for transport to other institutions. *Ex situ* conservation and research programs not only can prevent extinction through captive management and re-introduction to the wild, but offer opportunities for research to identify and, thus, protect key habitats (Zippel et al. 2011).

**Conclusion**

The species of the genus *Staurois* live and breed along fast-flowing streams and waterfalls. For the first time it was possible to *ex situ* breed two foot-flagging species in captivity and demonstrate the importance of fresh water streams and adjacent gravel pools for reproduction. We suggest that to successfully breed stream dwelling anurans with territorial males/females (also immature juveniles as mentioned previously) performing spacing behaviors (e.g., foot flagging), large and diversely structured terraria, including a waterfall and several options for egg deposition should promise the best success rate for future breeding programs. Further, we emphasize, that zoo-based conservations and research programs help to identify ecological factors that are necessary for the survival of threatened species, and also raise awareness to the ongoing amphibian decline. Public awareness of the conservation needs of threatened amphibian species through zoo-based conservation breeding programs may then be translated into in-range conservation initiatives by regional governments and local stakeholders who are also concerned with the *ex situ* conservation of these two species.

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**Author Contributions.**—DP carried out the study, analyzed pictures and available data and wrote the manuscript. AW participated in the design of the study and coordinated its implementations at the Vienna Zoo. TW designed and build the breeding facility, carried out the
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**Figure 10.** Juvenile *Staurois parvus* performing a foot-flagging behavior. Interdigital webbing are transparent grey and not white as observed in adults (see also Fig. 2). *Image by N. Potensky.*

**Figure 11.** Tadpoles of *Staurois guttatus*. *Image by N. Potensky.*
import of the species, and participated in all decision processes. WH conceived and coordinated the study. All authors read and approved the final manuscript.

**Literature cited**


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Doris Preininger has already worked with foot-flagging frogs in her undergraduate studies. In her graduation thesis she addresses the multimodal (acoustic and visual) communication in anurans and tries to explain how selection on senders and receivers promotes complex displays under different acoustic and environmental conditions. She is currently completing her dissertation at the Department of Evolutionary Biology, University Vienna. Her research includes foot-flagging species from Borneo and India and focuses on a bio-acoustic and experimental approach in the natural habitat of the respective species. In several visits to Borneo it became quite obvious to her that agricultural demands gradually degrade the primary forest and that every conservation effort possible should be immediately taken to conserve and protect the biodiversity of the rainforest.

Anton Weissenbacher is Zoological Curator at Vienna Zoo, committee member of the European Association of Aquariums and coordinator of the European StudBook (ESB) of *Brachylophus fasciatus*. At Vienna Zoo he is responsible for the zoological and technical management of the aquarium, the “Desert house,” the “Rainforest house,” and monitors all zoo issues concerning fishes, amphibians, reptiles, and invertebrates. Under his zoological guidance, the zoo has registered several exceptional breeding successes such as the world’s first Northern river terrapin, *Batagur baska*, hatched in captivity. Together with his team he manages the world’s largest *Aphanius* species breeding group. He has supervised various scientific publications and has initiated several conservation projects including Project *Batagur baska*.

Thomas Wampula has worked since 1996 at the Vienna Zoo Schönbrunn. He started as Animal Care Taker at the Aquarium-house and later transferred to the “Rainforest house” where his first and foremost interests were amphibians, reptiles, and fish. His duties and responsibilities included the arrangement and design of terraria and the maintenance of facilities. In 2007 he became a member of the Department of Technology and Project Development at the zoo and now is engaged in planning, design, and development of vivaria in the entire Vienna Zoo. The foot-flagging frog project has repeatedly led him to Borneo, where he assisted in field work, capture, transport, and care of frogs, and at the zoo he managed the construction of the breeding facility.
Walter Hödl has an international record in a wide range of topics in amphibian ecology and behavior. Since 1997 he has worked as an Associate Professor at the Institute of Zoology, University of Vienna. During the last years, he has studied numerous foot-flagging frog species in Asia, Australia, and South America and has established the South-East Asian frog genus *Staurois* spp. as a research model. Previous work on visual signaling frog species began more than 10 years ago, when he documented for the first time in a scientific film\(^1\)—together with Brazilian colleagues—anuran foot-flagging behavior, and later compared visual signal repertoires of anuran species worldwide. He discovered the use of the vocal sac as a visual signal independently of sound production in *Phrynobatrachus kreffti*, and set off a study on color change in the explosively breeding anuran species *Rana arvalis*. In the neotropics, his so called “handy fellow” *Allobates femoralis* has been his research focus for over the past 30 years and has led to numerous research and teaching visits to Brazil (Universities at Belém, São Luís João Pessoa, Manaus, São Paulo, Rio Branco, Ribeirão Preto, Feira da Santana, and at MPEG Belem, INPA Manaus) and Peru and French Guiana, enabling him to spend over eight years of fieldwork in Amazonia. Among many functions, he is a member of the scientific committee of WWF Austria and the head of the nature conservation society of lower Austria and continuously establishes cooperation around the globe to promote anuran research and conservation.