

A bioacoustic and morphometric account of Albertine Rift litter frog, *Arthroleptis schubotzi* (Amphibia: Anura)

H. Christoph Liedtke, Lund University, Sweden

Isabelle Maiditsch, University of Vienna, Austria

Jacob Ng'wava, National Museums of Kenya

Abstract

The biology of East African amphibians are poorly studied, one of which being *Arthroleptis schubotzi*, a small, direct developing frog, characterised with an extremely elongated third digit in males. This study revealed that male *A. schubotzi*, a diurnal leaf litter frog, calls from within the undergrowth at edge habitats in Kibale Forest, West Uganda. These anurans display a peak calling activity in the late afternoon. Morphological measurements indicate that the third finger is not necessarily allometrically scaled to body size and can reach four times the length of neighbouring digits. High site fidelity in males is evident and two calls, an advertisement and an encounter call are described here, both of which can be readily elicited via playback experiments. Possible proximate functions of the elongated digit in males are also discussed.

INTRODUCTION

Amidst the growing concern among the scientific communities that amphibian populations have been experiencing catastrophic declines globally over the last few decades (Gardner *et al.*, 2007; Houlahan *et al.*, 2000; Semlitsch, 2003 and Lips *et al.*, 2005a), the need for concerted efforts into studies on amphibian populations becomes inevitable. Amphibians represent some of the most endangered vertebrate taxa (IUCN 2004), alarmingly threatened more than either mammals or birds (Stuart *et al.*, 2004). In light of their sensitivity to environmental change, it is particularly important that we understand baseline patterns of amphibian diversity and habitat preferences in areas that still retain relatively intact habitat (Gardner *et al.*, 2007). Basic field data are necessary to monitor population declines, and evaluate impacts of different conservation options.

Great paucity still exists regarding data on tropical African amphibian populations and communities with the exception of South Africa (Passmore, 1976, 1977; Passmore & Carrunthers, 1995) and West Africa (Rödel, 1998, 2000, 2003; Rödel & Ernst, 2001). Otherwise little is known about the behaviour and ecology of frogs occurring in sub-Saharan Africa.

Our study area is situated in the Kibale Forest which like most of the other tropical forests is envisaged to have high diversity of both fauna and flora. Very few studies have however been undertaken on the amphibians of this forest (Vonesh 2001), and most of the existing information is

just from anecdotal collections. By the year 2001, a list of 29 species including *Arthroleptis schubotzi* had been generated for Kibale Forest National Park (Loveridge, 1942; Schiøtz, 1975; Vonesh, 2001).

The recently reclassified Albertine Rift litter frog *Arthroleptis schubotzi* NIEDEN 1911 (formerly *Schoutedenella schubotzi*) belongs to a group of squeakers in the family Arthroleptidae. The family consists of small forest dwelling frog species usually found associated with leaf litter, (Channing & Howell, 2006). Male *A. schubotzi* reach a length of 21 mm while maximum female size reaches 23 mm. The frogs have short legs and their tympanum whose size is half that of the eye is visible. The nostrils are closer to the snout tip than the eye. The inner metatarsal tubercle is very small and round. The belly is pigmented and a common pattern includes a dark spot on the head. Males have a black throat. These diurnal, cryptically coloured frogs are well adapted to life in the undergrowth showing direct development (i.e. with no free swimming tadpole phase), reproducing without the need of a permanent body of water.

Breeding males of many species in the family Arthroleptidae have an elongated third finger which amazingly may reach nearly half of the body length (Passmore & Carruthers, 1995) and has many spines on the inner surface (Channing & Howell, 2006). The proximate function of this elongated digit is not yet known, but Channing & Howell (2006) suggest that the long finger may have an important role in breeding, citing record of males protecting their calling site against other males by wrestling with intruders, using these long fingers. Other anecdotes suggest the finger is important for amplexus or even vibration of leaf litter for close-range communication (Blackburn, 2009).

While the vocalisations of this species have been reported as a harsh series of double chirps, analyses of the temporal and spectral call characteristics has not been analysed before (Channing & Howell, 2006).

While studies on *A. schubotzi* have primarily focused on its phylogeny, little is known about its behaviour and ecology. This study aimed at providing data on the animal's general ecology by investigating calling activity of males, calling site microhabitat and territoriality of males, but more importantly, experimental male-male and male-female encounters were planned to gather insight into the possible functions of the long finger in males. The above listed anecdotes (territorial wrestling, clasping position during amplexus and tactile communication) were intended to be tested. In addition, the call was recorded and formally described.

MATERIALS AND METHOD

Study site

The study was executed within and around the vicinity of the Makerere University Biological Field Station (MUBFS), in Kibale Forest National Park, a 766 km² reserve located in western Uganda (0°13'–0°41' N and 30°19'–30°32' E; Gillespie & Chapman, 2001) near the eastern base of the Ruwenzori Mountains. Kibale was established as a national park in 1993; prior to that it was managed as a forest reserve with selective timber extraction and a number of exotic softwood plantations. The park is characterised as primarily a moist evergreen forest, transitional between lowland rain forest and montane rain forest (Vonesh, 2001; Struhsaker, 1997; Skorupa, 1988), but a variety of habitats including swamp, grassland, woodland thicket, and colonising scrub are also represented (Struhsaker, 1997). Rainfall is typically concentrated during two distinct wet seasons, March through May and September through November. The mean annual maximum temperature measured at Kanyawara is 23.3 °C, and mean annual minimum temperature is 16.2 °C (Struhsaker, 1997). The investigation comprised eight days in mid August 2010, six of which were committed to field observations.

Study organism

Male *A. schubotzi* calling in the leaf litter were identified based on their calling activity and on morphological description provided by Channing & Howell (2006) and Spawls *et al.* (2006).

19 individuals were located, calling from the undergrowth at the perimeter of the Kibale Field Station during a pilot observation carried out after a brief rainfall prior to the commencement of the study (07.08.2010). Due to high calling activity and convenience of their location, these 19 individuals were chosen to be included in the study and their approximate location was marked (Figure 1). In the course of the study, six more calling males were identified. The call of one acoustically active male was recorded during the pilot study to be used as a playback source for stimulating less active males in subsequent experiments.

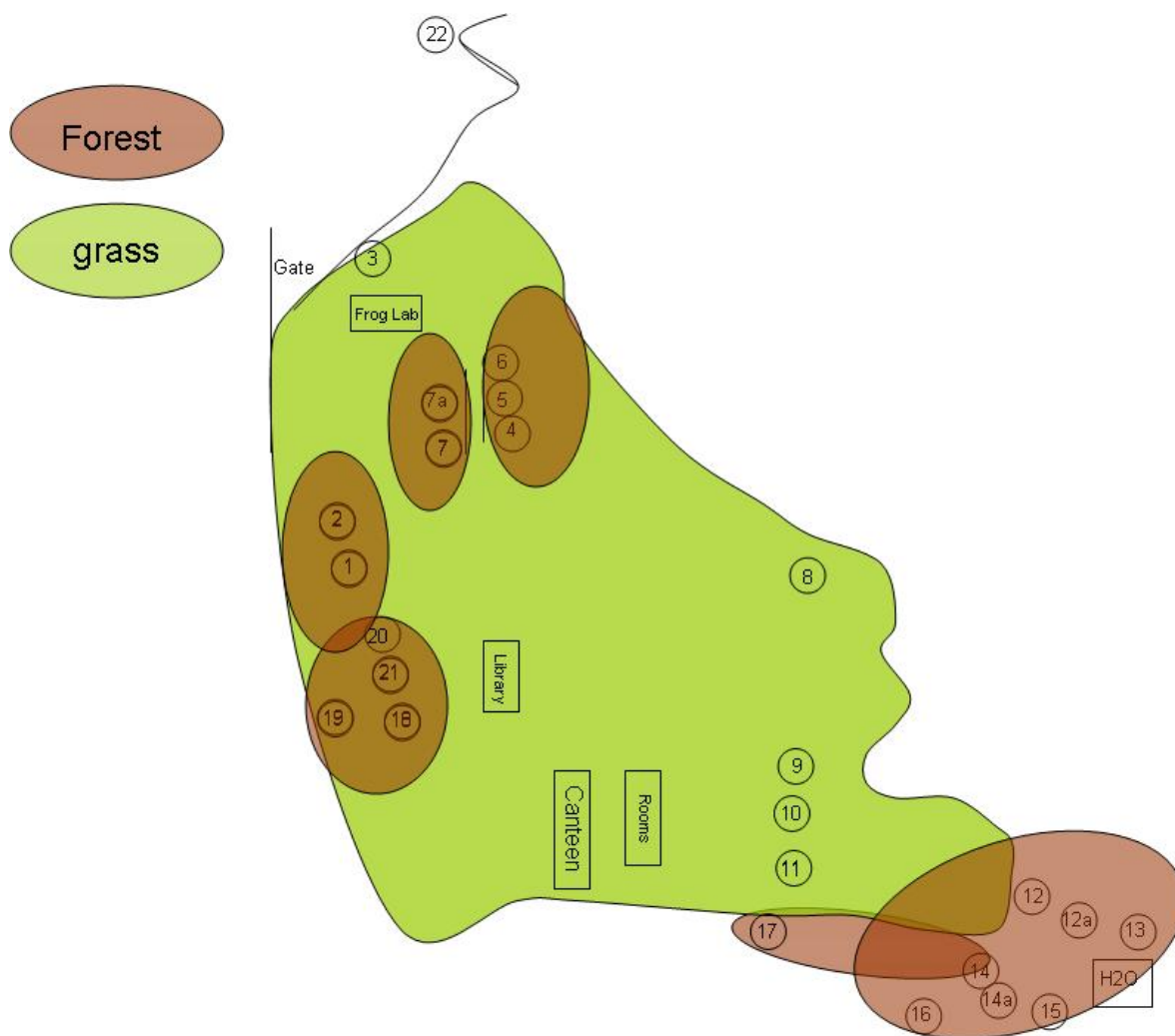


Figure 1. Map of calling sites of male *A. schubotzi* individuals around Makerere University Biological Field Station (MUBFS).

Daily calling activity

The calling activity of 25 individuals was monitored at three non-arbitrary times per day, at 08:00h, 14:30h and 18:00h between 14.8.10 and 19.8.10. These times were thought to best reflect the frogs' daily variation in activity based on a preliminary 24 hour observation period (13.8.10). For each observation period, activity was recorded in a binomial fashion, either "calling" or "not calling", for each individual. Acoustically inactive males were stimulated with playbacks of calls, to try to elicit a response from as many of the 25 males as possible. Temperature and relative humidity was recorded during each observation using an Environmental Meter (N09AQ, Precisions Gold).

Call description

In order to describe the call of *A. schubotzi*, both spontaneous calls and acoustic responses to conspecific playbacks (stimulated calls) were recorded with a Marantz recorder (Professional PMD660) and a directional transistor microphone (Sennheiser K6). In addition to the more frequent

advertisement call, a yet unknown call type in this species, the close-range encounter call was recorded, elicited by the frogs in close proximity to the playback source. All recordings were analyzed using Raven pro 1.4 (Cornel University, USA).

Morphological measurements

For the purpose of investigating allometric scaling effects of the enlarged third digit, calling individuals were caught by hand and taken to the laboratory for morphometric analysis. Frogs were sedated by applying a small dose of benzocaine on the back of the head and photographed (alongside a ruler) with a Nikon D5000 camera fitted with an AF-S Nikkor 18-105 mm, 1:3.5-5.6 G ED zoom lens. This method allows for more accurate measurements than using callipers on unsedated individuals. Snout-Urostyle-Length, length of all fingers and radius and humerus were then measured using ImageJ v1.41o (Wayne Rasband, National Institute of Health USA).

All frogs in captivity were accommodated in separate enclosures (20 cm x 20 cm) and provided with water and food (fruit flies attracted by pieces of banana) *ad libitum* and eventually returned to their exact point of capture.

Behavioural experiments

Although male-male and male-female interaction experiments were planned, these could not be sufficiently executed due to acoustic inactivity which was assumed to be associated with dry weather conditions. Trial experiments using video recordings and mirror tests were also executed, a qualitative account of which is given in the discussion.

Data analysis

The data were analysed using Microsoft Excel, Minitab and Raven pro 1.4 for sound analyses.

RESULTS

Daily calling activity

Over the study period of six days, 13% of all calling events were spontaneous calls, 11% of which were documented on the first day of observation. To standardise results, these 13% of data points were not included in the calling activity analysis.

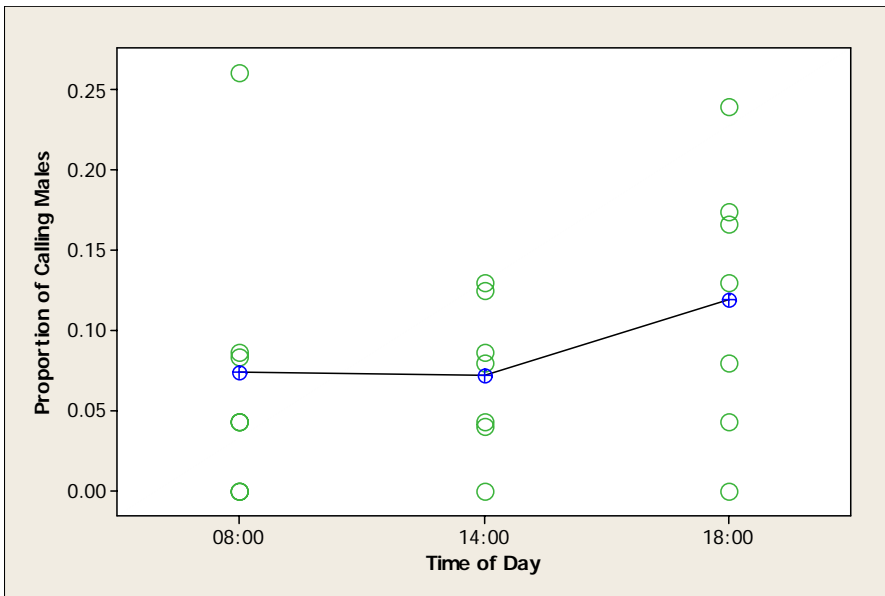


Figure 2. Individual plot of proportion of calling males at three time intervals over six days. Crosshairs represent mean proportions of calling males (ANOVA; $F_{2,18} = 0.87, p = 0.437$).

Overall calling activity was low with no more than 25% of all known males calling at a time throughout the six days of observations. The mean calling activity of males at different times throughout the day is not statistically significant (ANOVA; $F_{2,18} = 0.87, p = 0.437$), however the variance in the morning and evening is considerably greater than that at 14:00 (SD = 2.06, 1.11 and 2.04 respectively), with the highest calling activity throughout this study being recorded for these times as well.

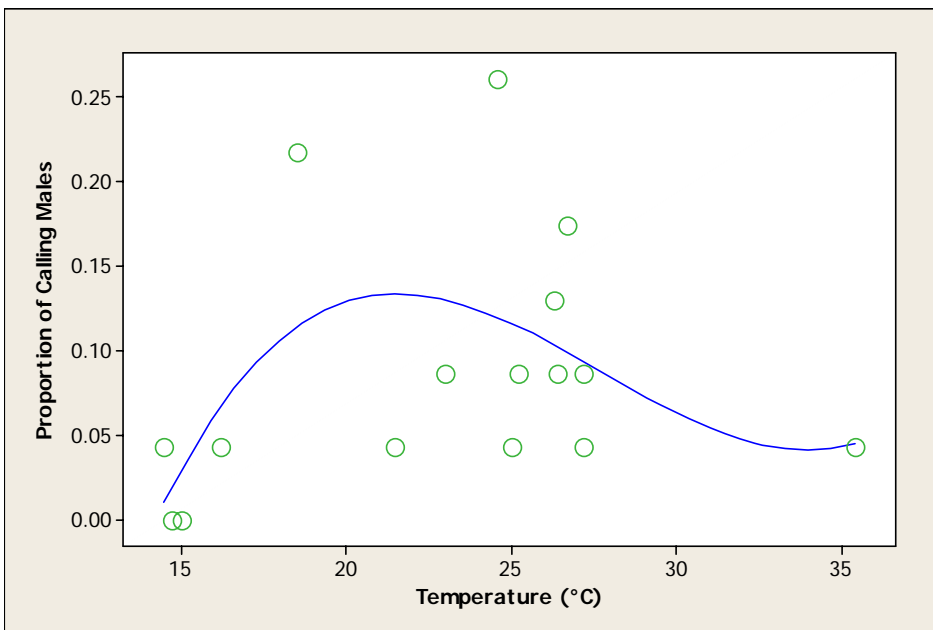


Figure 3. Scatter plot of proportion of calling males in relation to temperature.

Calling activity, when plotted against temperature, results in an asymmetric bell shaped distribution with the greatest calling activity recorded at 24.6 °C. Male *A. schubotzi* have a preferred temperature range between ca. 22 °C and 27 °C with data points clustering within that range. Furthermore, temperatures below this cluster experienced a greater calling activity than temperatures above this limit, which may be correlated with the maximum recorded daily activity being in the cooler mornings and evenings (Figure 2).

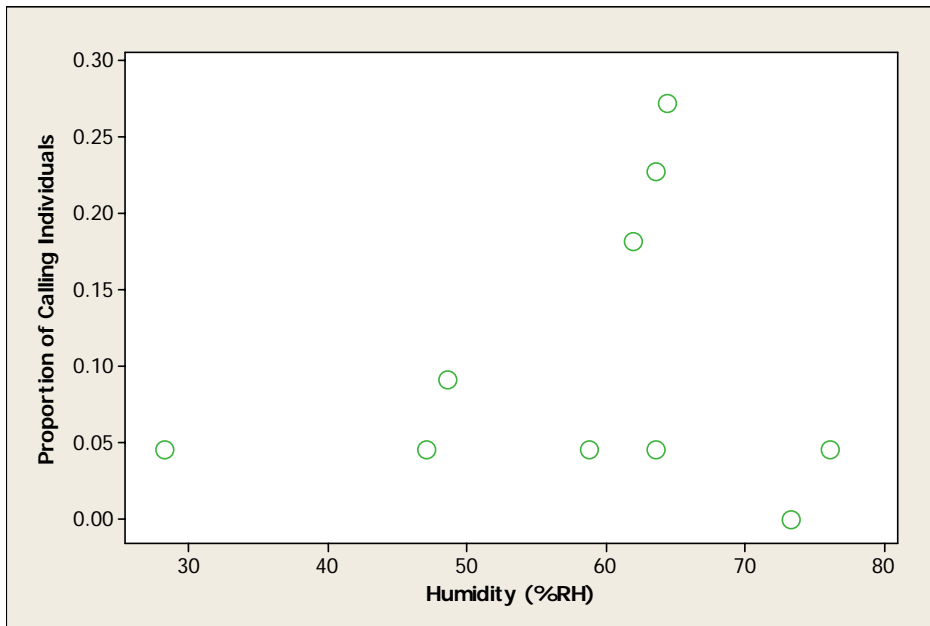


Figure 4. Scatter plot of proportion of calling males in relation to humidity.

The highest playback-induced calling activity was recorded at a humidity of 64.3 %RH and the general trend predicted is that calling activity increases with humidity (Figure 4). The low calling activity at the two highest recorded humidity levels are anomalies perhaps explained by being linked to extremely low temperature.

Morphological measurements

The four measured individuals boast a third finger longer than their fore- or upper arm and this elongated digit exceeds the size of the neighbouring finger up to four times in length (Figure 5a & b). Digits 1, 2 and 4 are of similar size, varying by less than one millimetre within individuals. The smallest individual (SUL = 20.7 mm) has the longest finger (8.24 mm) relative to body size indicating that this elongated digit may not be allometrically scaled to body size.

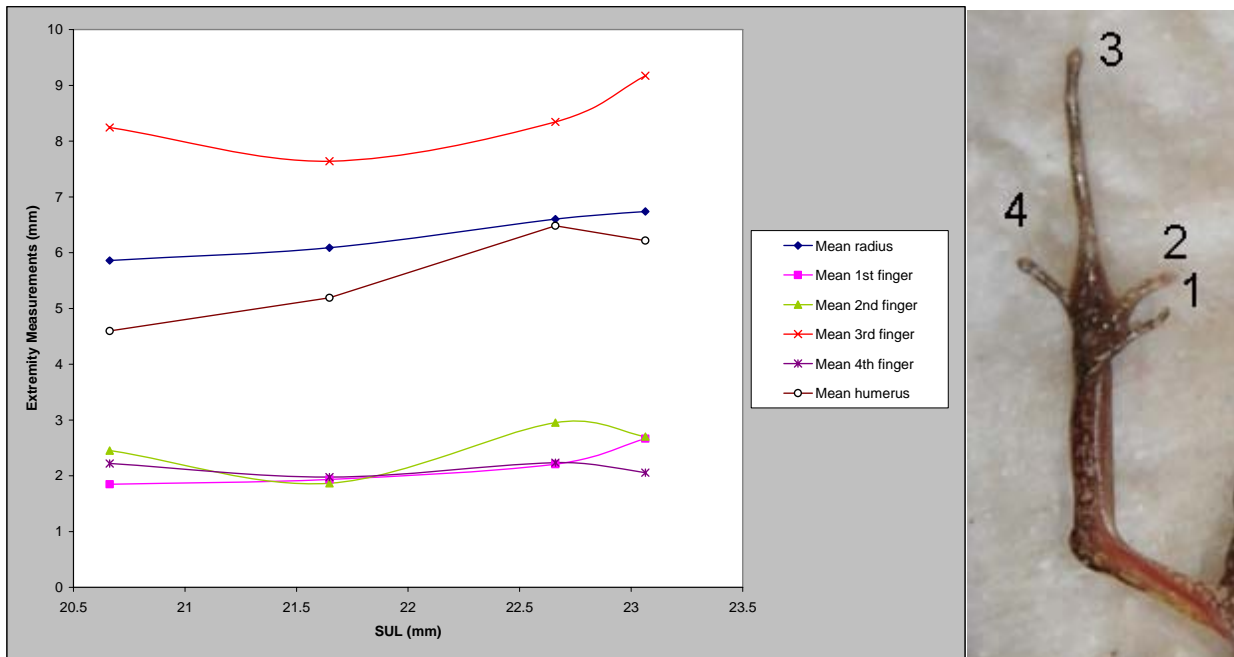


Figure 5. a) Length of extremities in relation to body size (Snout-Urostyle-Length) for four caught individuals. Values are means of left and right forelimb measurements. b) photo of left arm showing elongated third digit.

Acoustics

The vocalisations of many East African frogs, including *A. schubotzi* have not yet been described and it was therefore a central aim of this study to record and describe these vocalisations. The call is a high pitched metallic sound with a frequency of around 4800 Hz ($n = 7$, mean = 4694.52 Hz.). The call is characterised by a note duration of 0.017 sec., an internote duration of 0.081 sec., a call duration of 0.129 seconds and an inter-call duration i.e. the pauses between the calls, of 5.681 seconds (Figure 6).

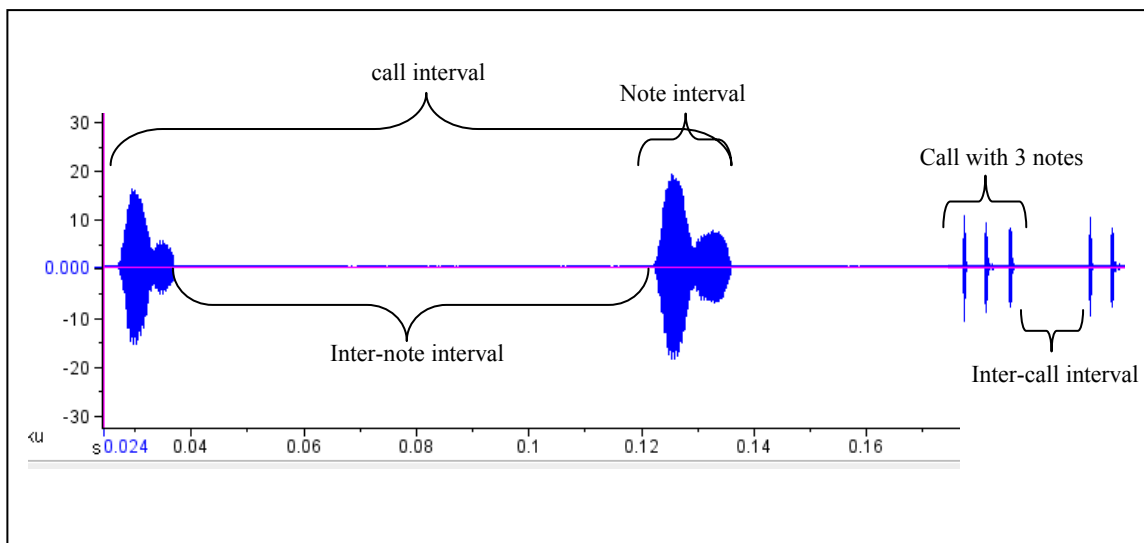


Figure 6. Two-note advertisement call and measurements for describing the call.

The spontaneous vocalisation shows a remarkable pattern consisting of repeated sets of 5 two-note calls followed by 14 three-note calls (repeated four times in one recording). To the best knowledge of the authors, such a regularly repetitive pattern is very rare in amphibians. Minimal variation existed in inter-note intervals with a standard deviation of 0.036 seconds.

It is important to note that there were differences between the spontaneous calls and the playback stimulated calls. Although three-note calls were more common in the spontaneous calls, two-note calls were more prominent in the stimulated calls. The dominant call frequency varied between 4300 and almost 4900 Hz across all individuals (Figure 7).

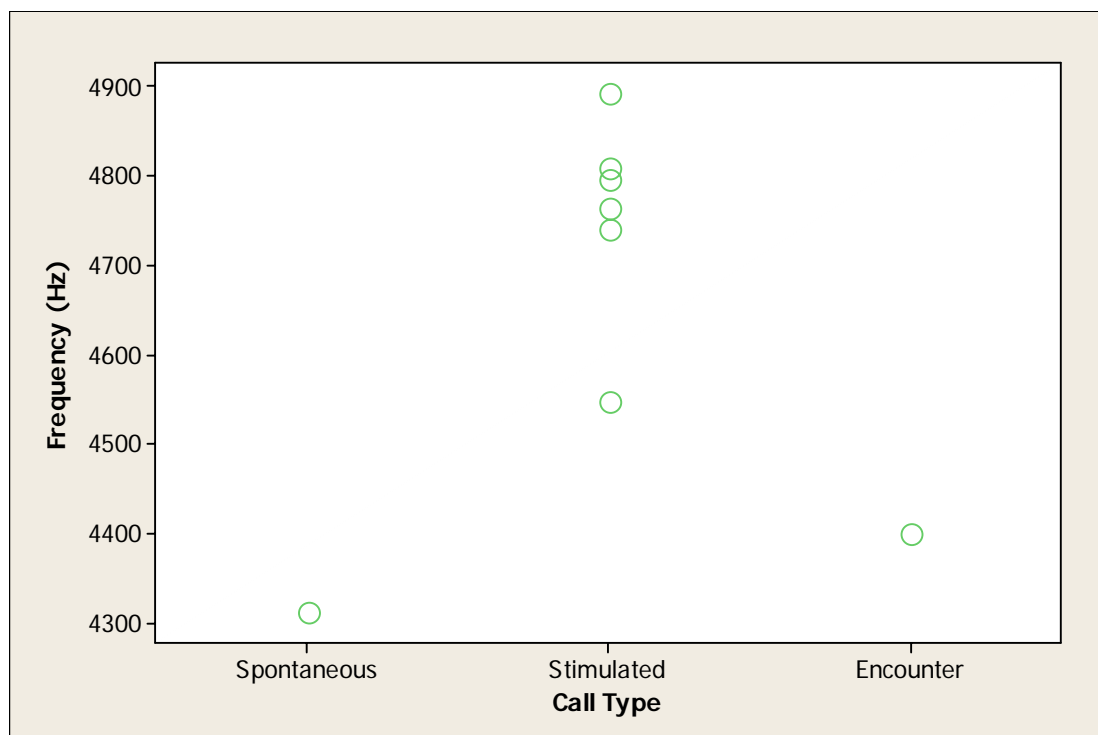


Figure 7. Dominant frequency of vocalisations of *A. schubotzi*.

The second call which was recorded during the observation was a close range encounter call. The males react strongly towards the speakers with a screech-like sound and the Oscillogram and Sonogram of this call look very different to those of the advertisement call. The notes in this call have a high variable pulse rate and a longer, lower pitched frequency compared to the advertisement call (Figures 8 & 9). The three-note encounter call is characterised by two notes having a very high pulse rate followed by a note similar to the repeated units in the advertisement call (Figures 8 & 9). The call is very variable, especially in relation to the pulses, the rate and the peak frequency of this call is 4399.39 Hz, the call duration is 0.129 seconds. The inter-note duration has a rate of 0.081 seconds and the note duration is 0.016 seconds.

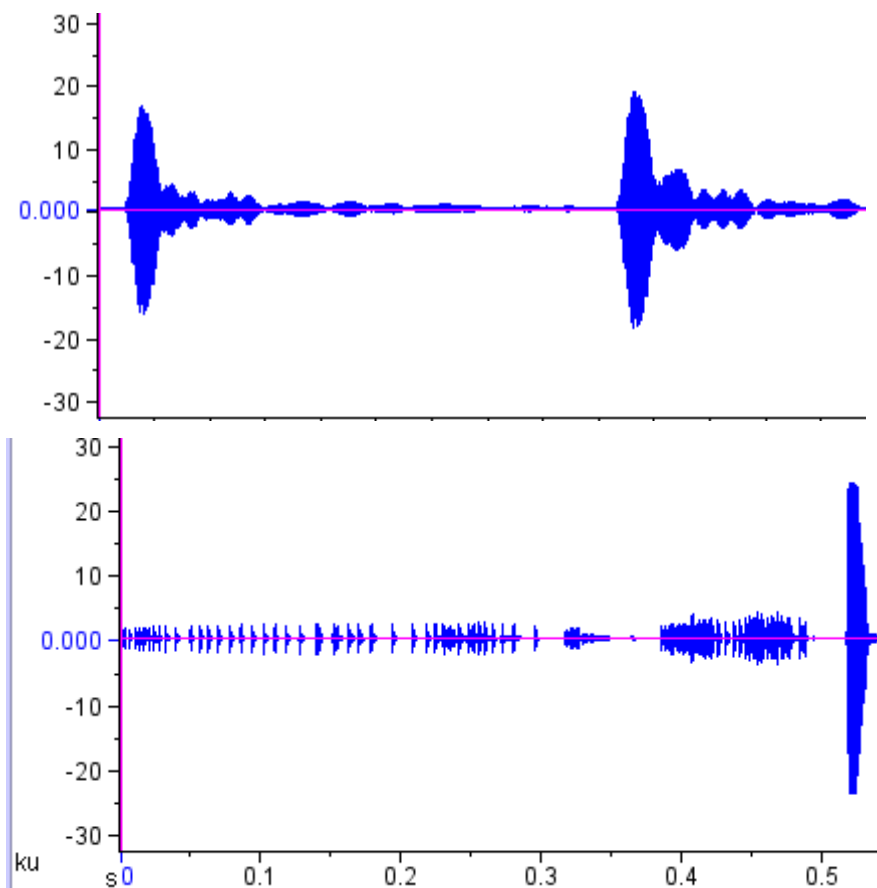


Figure 8. Oscillogram of the advertisement call (above: Peak Frequency 4694.52 Hz) and the encounter call (below: Peak Frequency 4399.39 Hz).

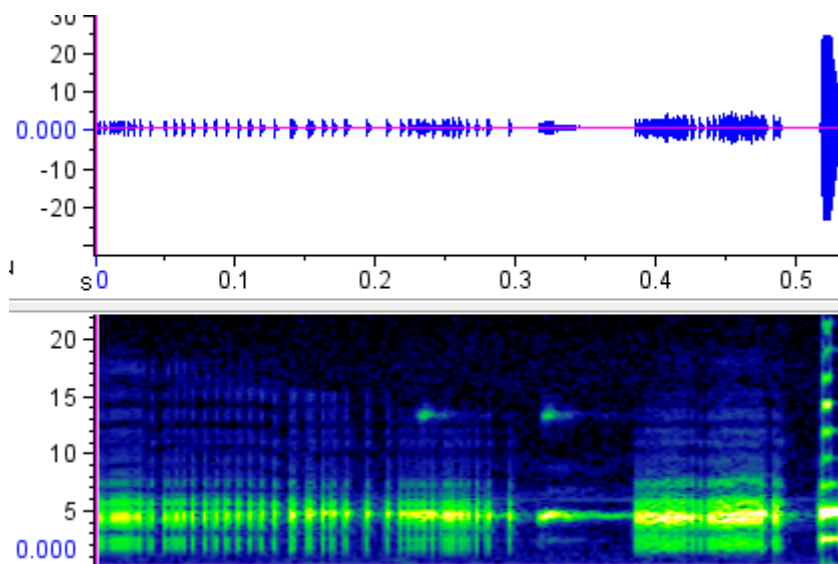


Figure 9. Oscillogram and Sonogram of the encounter call.

One of the pilot experiments, the antiphonal playback procedure, aimed to compare the calling rate of the male to a synthetic regular calling rate produced from a speaker. A regular playback was given, which had an inter-call interval time of two seconds. With two individuals the experiment could be

performed. The results did not show a regular calling pattern in the two focal males and thus no firm conclusions can be drawn. Both were calling several times in irregular order to the playback stimulus (Figure 10).

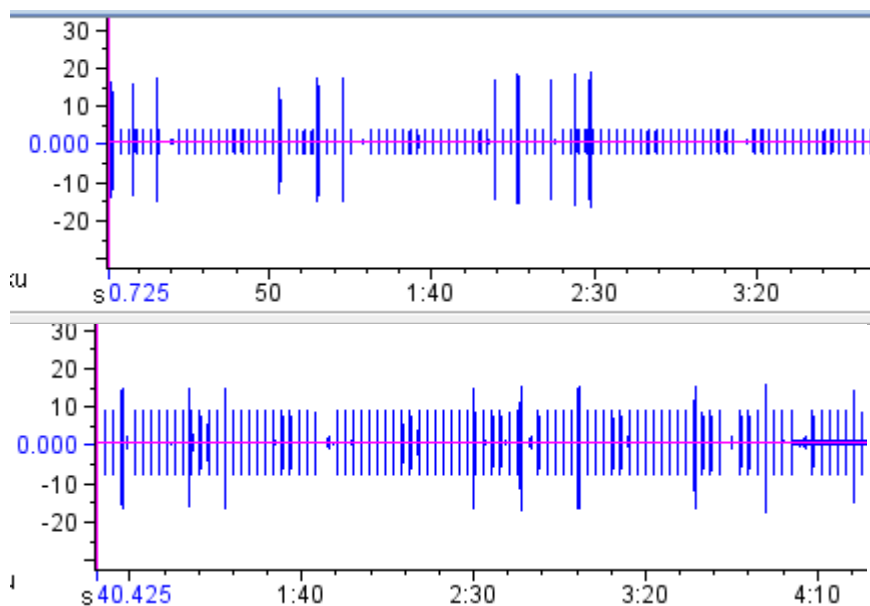


Figure 10. Oscillogram of the two recorded males in the antiphonal experiment (male 22 above and male 7a below).

DISCUSSION

Why do male *Arthroleptis schubotzi* boast a finger longer than its forearm? Despite having such a peculiar trait, little is known about the behaviour and ecology of this species. Claims of the finger playing a role in wrestling or tactile communication are cited in the literature (Blackburn, 2009) and have inspired this project, and the authors aimed to document scientifically possible proximate explanations for such a seemingly handicap trait. Unfortunately, MUBFS received no recorded rainfall during the six days of study and nearing the end of the dry season, one could speculate that this was a major explanatory factor for the low levels of anuran activity, hindering this project. Despite this setback, the study presents novel ecological and behavioural data for this species and more importantly, will hopefully draw further attention to answer the evolutionary puzzle of why *Arthroleptis schubotzi* has an unusually long finger.

Daily observations have revealed that the maximum calling activity of the Albertine Rift litter frog is in the early mornings and late afternoons, with low proportions of males responding to playback close to midday. Such a bimodal activity pattern is common in diurnal anurans and is comparable to the activity of dendrobatids of South and Central America (Hödl *et al.*, 2004). The bimodal calling activity correlates well with temperature preferences as the highest activity of *A. schubotzi* was recorded between 22 °C and 27 °C reflecting evening and morning conditions. Although there were

small proportions of males calling at lower temperatures (experienced in some cooler mornings), few called at higher temperatures such as those recorded in the early afternoons. The data on humidity levels and calling activity suggests there is a positive correlation between the two. If a large enough sample size were available, a multivariate regression analysis, combining the effect of temperature and humidity on frog activity would be a very appropriate analysis and would allow for more complete picture when predicting trends, as these two environmental factors are often linked.

Site fidelity strongly suggests territoriality in male *A. schubotzi*. Over the six days of observations, males were calling from the same localities and when stimulated with playbacks of advertisement calls, responded readily and approached the speaker when placed within a 2 m distance of their location. The advertisement call consists of two or three short, high pitched metallic chirps and occasionally single notes are given. A second call type, termed close range encounter call is also documented here for this species, produced by focal males once within hopping distance to the playback source. Whether an encounter with a calling male in such a scenario then descends into physical or ritualistic combat where the elongated finger has been observed to play a role (Blackburn, 2009) could not be determined in this study. Simulations using mirrors with captive males did not yield any response either, one explanation for this being that males in captivity may have been stressed or no longer displaying agonistic behaviour.

Morphological measurements revealed that the third finger in males is up to four times as long as the other fingers and although no concrete conclusions can be drawn about allometric scaling and the degree of individual variation, it is worth noting that the smallest male bore the longest finger relative to body size. Whether this trait experiences selective pressures via female choice could again not be tested as the number of active males was low and no females were sighted throughout the study period.

Males in captivity were observed to bury themselves by alternating hind leg movements into moist soil, which may be an adaptation to avoid desiccation. After handling, males were also observed to undergo rigorous wiping behaviour. Individuals covered with sand again displayed this behaviour and cleaned themselves by moving the arm upwards and brushing the elongated finger forward across the head and over the eyes. Although the elongated third digit appears to give males a clear advantage for this purpose Channing & Howell (2006) reports this trait to be absent in females and assuming strong similarities in the niche exploitation of both sexes, such an adaptation should then be present in females as well. It therefore seems unlikely for grooming to be the primary function of this sexually dimorphic trait.

Although personal observations conclude that the finger is often bent backwards and appears to be a hindrance to movement in the frog's natural environment, qualitative analysis of slow motion videos of caught individuals walking and hopping on a uniform surface does not support this claim and merits more attention into the possible handicap this trait may cause in terms of free movement or predator evasion.

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