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Advertisement call and notes on the ecology of *Afrixalus orophilus* (Anura: Hyperoliidae) in Rwanda

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The genus *Afrixalus* Laurent, 1944 (Hyperoliidae) is represented in Rwanda by three species. Whereas *A. quadrivittatus* (Werner, 1908) is widespread in open habitats at lower elevations below 2000 m a.s.l., including natural savanna and farmland (Laurent 1982, Sinsch et al. 2012, Dehling & Dehling in press), the other two species, *A. orophilus* (Laurent, 1947) and the recently described *A. phantasma* Dehling, Greenbaum, Kusamba & Portik, 2022 in Greenbaum et al. (2022), are restricted to montane forest and grassland at higher elevations in Rwanda (Laurent 1982, 1983, Greenbaum et al. 2022). Although *A. orophilus* has been reported to be common in montane regions of Rwanda and Burundi and to be often found in rather open places (Laurent 1983), it has actually been collected at only four localities in Rwanda (Laurent 1950, 1982). Nowadays, it is the most rarely observed species of the genus in the country, with very few recent observations. *Afrixalus orophilus* was originally described from the Virunga National Park in the North Kivu Province in eastern Democratic Republic of the Congo (Laurent 1947), with most of the paratypes originating from surrounding areas in the DRC and one from Rwanda (Gihorwe, 2400 m a.s.l., Karisimbi). The species was later also recorded from Burundi and from southwestern Uganda (Laurent 1982, Drewes & Vindum 1994). Little is known about its ecology. Laurent (1982) reported that it was found in various habitats in the Democratic Republic of the Congo, including reeds, coffee plantations, bamboo, ponds in sclerophyllous forest and, in the high valleys of Rwanda, in papyrus.

We herein describe for the first time the advertisement call of *A. orophilus* and provide some ecological information on a population in Rwanda. We observed the species at two ponds in close proximity to each other in a seasonally flooded meadow in an agricultural area at Rusumo (01°25' S, 29°50' E, 2048 m a.s.l.; WGS 84) at the northern end of the Rugezi wetland in northern Rwanda on 18 March 2022 between 18:30 and 20:00 h. Several dozen males were calling at the two sites from reeds between 0.5 and 2 m above the water surface. Ambient temperature was 17.6°C. The high activity was apparently caused by heavy rain during the preceding afternoon. In contrast to that, we had been unable to detect the species at the same site two weeks earlier during drier conditions. The advertisement calls were recorded as uncompressed files in WAVE format with a Sony PCM–D50 Linear PCM Recorder with built-in stereo microphones (Sony Deutschland GmbH, Cologne). For the characterisation of the advertisement call, we analysed a total of 55 calls from ten different males. Stereo recordings were converted to mono at a sampling rate of 44.1 kHz and 16 bits resolution using Adobe Audition 1.5. Spectrograms and waveforms were obtained applying Blackman-Harris Fast Fourier transformation with a FFT window width of 1024 points. Temporal data were

obtained from the waveforms and frequency information was obtained from the spectrograms and power spectra. Values are given as mean ± standard deviation with range in parentheses. Definitions of acoustic parameters follow Köhler et al. (2017). For comparison, we analysed recordings of vocalizations of *A. lacteus* Perret, 1976 and *A. weidholzi* (Mertens, 1938) from Cameroon as provided by Amiet & Goutte (2017) as well as own call recordings of *Afrixalus* species from Rwanda and Gabon. Additional data on the advertisement calls of *Afrixalus* species used for comparison were taken from the literature (Schiøtz 1999, Köhler et al. 2005, Amiet & Goutte 2017).

The observed specimens were unequivocally assigned to *A. orophilus* for showing the following morphological characters: small size, snout–vent length < 25 mm; dorsum smooth; dorsal colouration light brown with two slightly darker but conspicuous longitudinal narrow bands that converge on the head (Fig. 1); hand and foot webbing barely developed. Thereby, they morphologically match a large series of vouchers of *A. orophilus* from Rusumo, Rwanda, deposited in the collection of the Royal Museum for Central Africa, Tervuren, Belgium (RMCA 75-19-B-1291–1319) as well the holotype (RMCA 53016), all examined by the first author.

The advertisement call of *A. orophilus* typically consisted of 11.6 ± 1.7 (9–15) pulsed notes (Fig. 2). Depending on the number of notes, calls lasted between 462 ms (nine notes) and 841 ms (fifteen notes), with 619 ± 75 ms on average. A single call was exceptionally brief (275 ms), consisting of only five notes. Note repetition rate within the call was highest between the first two notes with 22.4 ± 2.4 (18–25) notes/s, and lowest between the last two notes of the call, with 17.1 ± 1.5 (14–18) notes/s. Overall note repetition rate was 20.3 ± 3.5 notes/s. Mean note duration was 29.2 ± 7.6 (20–44) ms. Note duration decreased from the first note (35.9 ± 4.4 [31–44] ms) to the last note (22.6 ± 1.7 [20–25] ms). The number of pulses per note was in most cases not determinable because pulses appeared to be partly fused, especially towards the end of the note. It is unclear if this was a result of echo effects or the actual amplitude structure. Few notes, however, consisted to their full extent of 5–6 discernible distinct pulses. Pulse repetition rate within notes was 250 pulses/s without measurable variation. Amplitude modulation was prominent within individual notes, with pulses in the middle of the notes having a higher relative amplitude than the pulses at the beginning and end of the note. Overall amplitude was lower in the first notes of the call (Fig. 2). Dominant frequency slightly increased from the first note, with 3081 ± 84 (2900–3180) Hz, to the last note of the call, with 3230 ± 71 (3090–3280) Hz. In few recordings made from short distance to the calling male, additional frequency bands were traceable at about 6400 and 9600 Hz. Prevalent bandwidth was 2700–3600 Hz. The calls were repeated in series at a variable rate of 9–36 calls per minute. The intervals between call series lasted up to several minutes.

In a recently published phylogeny of Central African *Afrixalus* species, *A. orophilus* was recovered in a weakly supported clade that included the Central and West African species *A. weidholzi* and *A. vibekensis* Schiøtz, 1967 (Greenbaum et al. 2022). The advertisement calls of these two species differ markedly from the advertisement call of

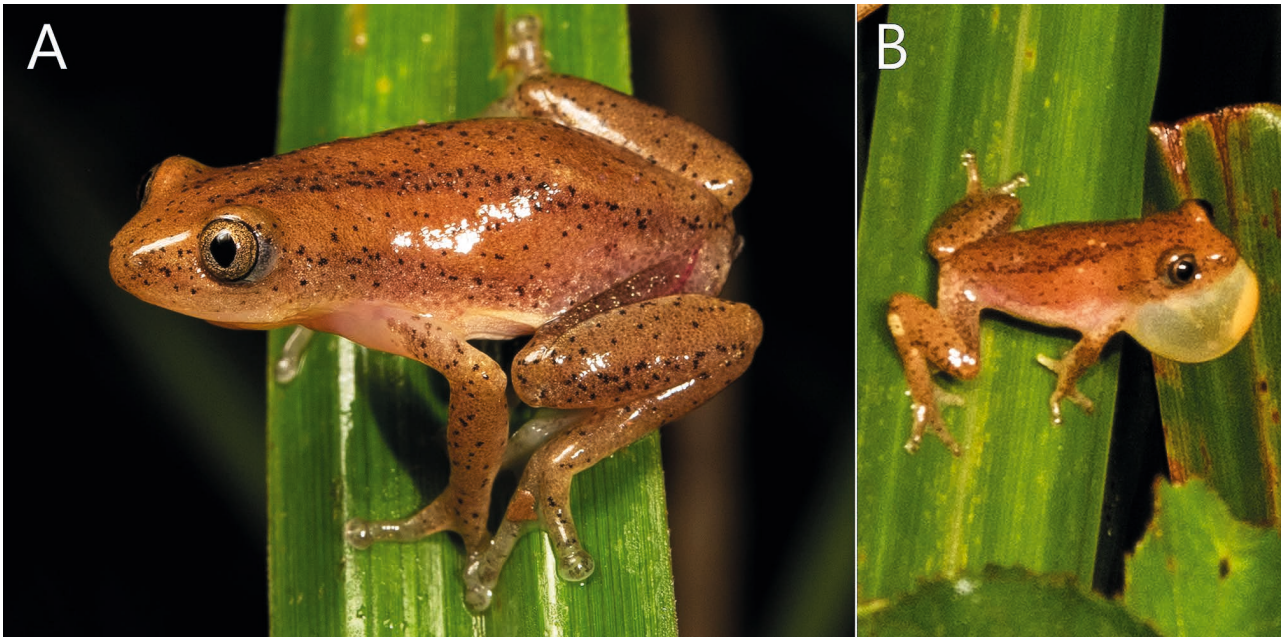

Figure 1. Adult males of *Afrixalus orophilus* from Rusumo, Rwanda, in life. (A) Non-calling male, showing nocturnal colouration. (B) Calling male in typical posture. Note the subgular vocal sac and the bright yellow colour of the disc-shaped gular gland. Photos by J. M. Dehling.

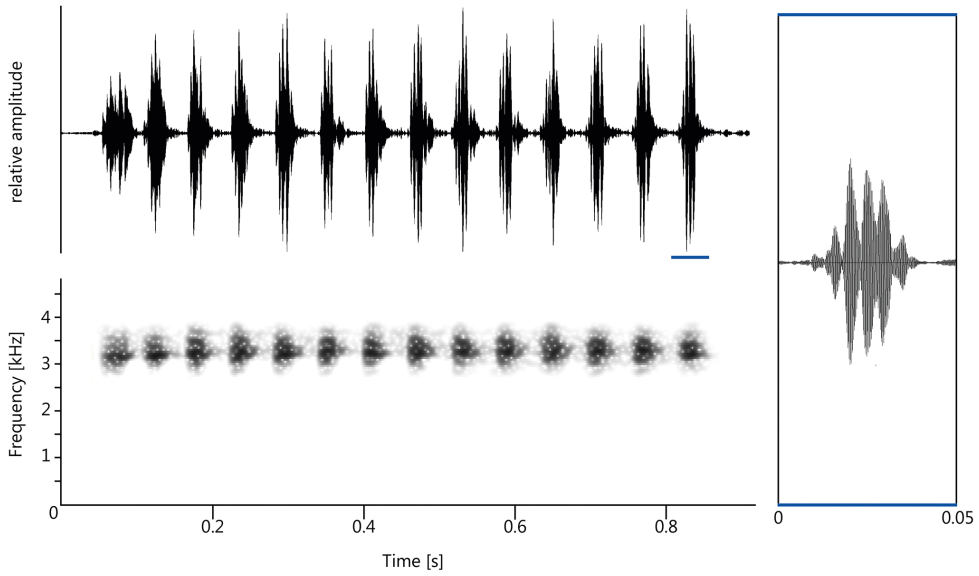

Figure 2. Waveform (top) and corresponding audiospectrogram (bottom) of a fourteen-note advertisement call of a male *Afrixalus orophilus* at Rusumo, Rwanda (ambient temperature 17.6°C). The inset on the right shows a waveform of the note marked by the blue bar in higher temporal resolution.

A. orophilus. The call of *A. vibekensis* consists of 27 notes (vs. 9–15 in *A. orophilus*) that are repeated at a rate of 28–32 notes/s (vs. 17–22 notes/s), lasts about 950 ms, and has a dominant frequency at about 5800 Hz (vs. 2900–3280 Hz) (see Schiøtz 1967). The advertisement call of *A. weidholzi* lasts up to 4100 ms, consists of up to 198 notes that are repeated at a rate of 35–47 notes/s, and has a dominant frequency at 4500–5000 Hz (see Schiøtz 1967, Rödel 1996, Amiet & Goutte 2017).

The advertisement call of *A. orophilus* most closely resembles the advertisement calls of the following species: calls of *A. osorioi* (Ferraira, 1906) consist of 8–19 notes, repeated at a rate of 14.5–30.0 notes/s, have a total call duration of 375–1122 ms, and a dominant frequency at 2500–3200 Hz; they differ by a slightly shorter note duration of 18–23 ms (vs. 20–44 ms in *A. orophilus*) (see Schiøtz 1975, 1982, Channing 2001, Köhler et al. 2005); calls of *A. lacteus* consist of 8–11 notes, repeated at a lower rate of about 11.5 notes/s (vs. 14–25 notes/s), total call duration is 760–967 ms, and dominant frequency is slightly higher at 3300–3400 Hz (vs. 2900–3280 Hz) (see Amiet & Goutte 2017); and calls of *A. phantasma*, consist of 5–6 notes (vs. 9–15) that are repeated at a lower rate of 7.1–11.7 notes/s (vs. 14–25 notes/s), total call duration is 388–620 ms, and dominant frequency is slightly higher at 3020–3810 Hz (vs. 2900–3280 Hz) (see Greenbaum et al. 2022).

Although Laurent (1982) reported that *A. orophilus* was found in papyrus, we did not find the species during repeated visits of other locations in the Rugezi wetland with extensive stands of papyrus at different times of the year. *Afrixalus orophilus* has been recorded recently in Rwanda only from the area around Rusumo. Most of the localities from where the species had been reported earlier (Laurent 1983) have been altered and converted to farmland. The observation that *A. orophilus* can use ponds in an agricultural area, although at the edge of a large natural wetland, raises hope that the species can cope with habitat alteration at least to some extent. The lack of recent records is probably the result of both limited field work in potential habitats and low detectability of the species in the field under non-optimal conditions. Systematic assessment of potential habitats at higher elevations in northern and western Rwanda and close monitoring of the Rusumo population will be necessary to appraise the current status of the species in the country.

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