

## Article

# A New Miniature Species of *Arthroleptis* (Anura: Arthroleptidae) from Nyungwe National Park, Rwanda <sup>†</sup>

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<sup>†</sup> New species: urn:lsid:zoobank.org:act:E8F25561-AD7C-46D8-9107-73F6ED10799C.

**Abstract:** A new, very small-sized species of *Arthroleptis* is described from western Rwanda. The new species occurs locally endemic in Nyungwe Forest and Cyamudongo Forest, where it inhabits the leaf litter of montane forests between 1800 and 2200 m a.s.l. It differs from all other members of the genus by a unique combination of morphological characters, including size (SVL of adult males 16.0–16.5 mm), skin texture, length of hindlimbs, and ventral colour pattern; by characteristics of its advertisement call that consists of a single note lasting  $17.4 \pm 6.4$  [11–32] ms and has a dominant frequency of  $5861 \pm 188$  [5531–6029] Hz; and also in the sequence of the 16S rRNA gene that differs from available homologous sequences of other species of the genus by an uncorrected p-distance of at least 4.6%. Details of the natural history as well as two additional call types are described.

**Keywords:** amphibians; diversity; bioacoustics; phylogenetics; *Arthroleptis schubotzi*; Nyungwe Forest; Cyamudongo Forest

## 1. Introduction

The genus *Arthroleptis* Smith, 1849 contains about 48 species that are distributed in almost all parts of sub-Saharan Africa [1,2]. There are several taxonomic problems and disagreement with respect to the validity of particular species and probably a number of cryptic species are currently unrecognized [3–7]. Historically, several small-sized members of the genus had been placed in a separate genus, *Schoutedenella* de Witte, 1921 [3,4,8,9], but the validity of the genus had not been accepted by most authors (e.g., [5,10,11]). Phylogenetic analyses have repeatedly demonstrated that small-sized *Arthroleptis* species considered to belong to *Schoutedenella* do not form a distinct clade separated from larger species and *Schoutedenella* is now widely recognized as a junior synonym of *Arthroleptis* [6,12]. A number of small *Arthroleptis* species have been described from the mountains and the graben valley of the Albertine Rift [3,8,13–16], and three species of *Arthroleptis* have been recorded from Rwanda [17]. *Arthroleptis adolfifriederici* Nieden, 1911 was described from the “Bugoiwald” and the “Rugegewald” (nowadays Gishwati and Nyungwe Forest, respectively) in Rwanda and is easily identified by its large size, smooth dorsum and long and slender limbs [13,17]. *Arthroleptis schubotzi* Nieden, 1911 has its type locality at “Usumbura” (=Bujumbura, Burundi) and was later also recorded from southwestern Tanzania, eastern Democratic Republic of the Congo, and western Rwanda [8,13,15,17–19]. It is a small-sized species (SVL of male and female < 24 mm) with a granular dorsal skin, a grey venter with blackish network and reddish thighs [17]. I repeatedly collected another very small species of *Arthroleptis* in Nyungwe and Cyamudongo Forest (*Arthroleptis* sp. [17]) with shagreened dorsal skin and a yellowish brown ventral colouration with yellow longitudinal stripes. Herein, I present the results of morphological, bioacoustics, and genetic comparisons of this species with its congeners and address its taxonomic status.



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## 2. Materials and Methods

**Collection of specimens and comparative data:** Specimens of *Arthroleptis* examined for this study (Table A1) were collected in Rwanda on several trips between September 2010 and December 2018. Specimens were collected by hand, photographed in life, and euthanized using a chlorobutanol solution. Tissue samples for molecular analyses were taken from liver or thigh muscle and stored in 98% ethanol. Specimens were fixed and stored in 70% ethanol and are deposited in the herpetological collection of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK), and Staatliches Museum für Naturkunde, Stuttgart, Germany (SMNS), and the working collection of the author (JMD). Comparative material (Table A1), including the types of most of the relevant species, was examined in the Royal Museum for Central Africa, Tervuren, Belgium (RMCA), and the Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung an der Humboldt-Universität zu Berlin, Germany (ZMB).

**Morphological examination:** I took the following measurements from specimens with a digital calliper (to the nearest 0.1 mm): snout-vent length (SVL, from tip of snout to vent); head length (HL, distance from posterior end of jaw to tip of snout); head width (HW, measured at widest point); horizontal eye diameter (ED); horizontal tympanum diameter (TD); snout length (SL, distance between anterior margin of eye and tip of snout); eye-to-nostril distance (EN, distance between anterior margin of eye and centre of nostril); nostril-to-snout distance (NS, distance between centre of nostril and tip of snout); internarial distance (NN, distance between centres of nostrils); intereye distance (EE, distance between anterior margins of eyes); upper eyelid width (EW, measured at widest point); interorbital distance (IO, shortest distance between upper eyelids); tibiofibula length (TFL, measured with both knee and tibio-tarsal articulation flexed); thigh length (THL, from knee to vent with thigh held perpendicularly to median body plane); foot length (FOL, distance from proximal end of inner metatarsal tubercle to tip of fourth toe); hand length (HND, measured from proximal end of inner metacarpal tubercle to tip of third finger); length of third finger (3FL, from metacarpus to tip of third finger). Additionally, I recorded the following qualitative characters: size and shape of thenar and palmar tubercles; presence of maxillary, premaxillary and mandibular teeth; presence of vomer ridges; shape of supratympanic fold; presence, extent and shape of dermal ridges of dorsum. Sex of males was determined by presence of transparent skin in the gular region and elongate third fingers, and examination of the gonads. Sex of females was determined by presence of ova. Data on colour pattern were obtained from specimens photographed in life. Additional data on morphological characters used for comparison were taken from [1,3,5,7,8,13–16,20–22].

**Phylogenetic analyses:** DNA was extracted from the tissue samples using Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Polymerase Chain Reaction (PCR) was used to amplify a fragment of approximately 550 base pairs of the 16S mitochondrial rRNA using standard primers: 16SAL (50-CGCCTGTTTACTAAAAACAT-30), and 16SBH (50-CCGGTCTGAACTCAGATCACGT-30). Amplification followed the standard PCR conditions [23] with the following thermal cycle profile: 120 s at 94 °C, followed by 33 cycles of 94 °C for 30 s, 49 °C (12S)/53 °C (16S) for 30 s, and extension at 65 °C for 60 s. All amplified PCR products were verified using electrophoresis on a 1.4% agarose gel stained with ethidium bromide. PCR products were purified using High pure PCR Product Purification Kit (Roche Diagnostics, Rotkreuz, Switzerland). Sequencing of both strands was performed with the DYEnamic ET Terminator Cycle Sequencing Premixkit (GE Healthcare, Munich, Germany) for sequencing reactions run on a Mega-BACE 1000 automated sequencer (GE Healthcare). Chromas lite 2.1.1 software (Technelysium Pty Ltd., Brisbane, Australia) and read the chromatograms of the sequences. The obtained sequences were compared with those in GenBank using a standard nucleotide-nucleotide BLAST search. The homologous sequences most similar to the sequences of the Rwandan species of *Arthroleptis* were downloaded and incorporated in an alignment. A sequence of *Leptopelis karissimbensis* from Rwanda was used as an outgroup. Sequences used in the study are listed in Table A2. The sequences were aligned using

the MUSCLE algorithm [24] implemented in MEGA version X [25]. The final alignment contained 24 sequences and was 563 bp in length. Pairwise distances were calculated in MEGA version X. The general time-reversible model with proportion of invariable sites and gamma-distributed rate variation among sites (GTR + I + G) was chosen as the best-fitting model of sequence evolution on the basis of the Akaike information criterion as implemented in jModelTest 2 [26] and was applied in a maximum likelihood (ML) analysis, performed in MEGA version X with heuristic searches with stepwise addition and TBR branch-swapping algorithm, generating 1000 bootstrap replicates.

**Bioacoustics:** 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, Germany). For the characterisation of the advertisement call, I analysed a total of 37 calls from six 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  $\pm$  standard deviation with range in parentheses. Definitions of acoustic parameters follow [27].

### 3. Results

Morphological, bioacoustic, and phylogenetic comparisons revealed that the small *Arthroleptis* species from Nyungwe cannot be assigned to any of the described species, nor any of the taxa considered synonyms. Therefore, it is herein described as a new species.

#### *Arthroleptis nyungwensis* sp. nov.

Nyungwe Squeaker

urn:lsid:zoobank.org:act:E8F25561-AD7C-46D8-9107-73F6ED10799C

*Arthroleptis* sp.—Dehling & Sinsch 2023: 13 [17].

**Holotype:** ZFMK 104075, adult male, from a small stream near Rukuzi (2.4635 S, 29.2293 E; 2200 m), Nyungwe National Park, Western Province, Rwanda, collected on 17 September 2014 by J. M. Dehling.

**Paratypes:** ZFMK 104076, adult male, from the type locality, collected in March 2013 by J.M. Dehling; ZFMK 104077, adult male, from Cyamudongo Forest (2.546 S, 28.989 E, about 2000 m), Western Province, Rwanda, collected 21 October 2018 by J.M. Dehling; SMNS 15743 and 15744, two subadult males, from the edge of Cyamudongo Forest (2.5378 S, 29.9942 E; 1830 m), Western Province, Rwanda, collected 14 June 2014 by J.M. Dehling.

**Referred material:** JMD 599, adult male, from the Kamiranzovu Waterfall Trail, appr. 1800 m a.s.l., Nyungwe National Park, Western Province, Rwanda, collected 25 September 2010 by J. M. Dehling.

**Diagnosis:** The species is assigned to the genus *Arthroleptis* for exhibiting the following morphological characteristics [28]: pedal webbing absent, median dorsal skin raphe present, third finger elongate in adult males, dermal spines present on third finger. The new species differs from all other members of the genus by the combination of the following characteristics: small size (SVL of adult males 16.0–16.5 mm); dorsal surfaces of head, trunk, and limbs and lateral surfaces of trunk finely shagreened; tympanum well visible externally; median lingual process absent; legs long, tibiotarsal joint reaching to eye; tibiofibular length equal to foot length; tip of third finger and tips of toes slightly enlarged; inner metatarsal tubercle very small and rounded, outer metatarsal tubercle absent; ventral colour reddish to orange with two slightly concave longitudinal yellow stripes and white dots; advertisement call consisting of single note, lasting  $17.4 \pm 6.4$  [11–32] ms and dominant frequency at  $5861 \pm 188$  [5531–6029] Hz; sequence of the 16S rRNA gene differing from available homologous sequences of other species by uncorrected p-distance of at least 4.6%.

**Description of the holotype:** Measurements of the holotype are provided in Table 1. Very small (SVL 16.5 mm) adult male; body slender, widest at temporal region, slightly tapering to groin (Figure 1A,B); limbs slender; head large (HL/SVL 0.37, HW/SVL 0.33),

longer than wide (HW/HL 0.89); snout moderately long (SL/HL 0.41), wider than long (SL/EE 0.79); rostral tip rounded in both dorsal view and lateral view; canthus rostralis moderately distinct, straight between eye and nostril; loreal region oblique; nostrils rounded, directed laterally, situated about halfway between eye and tip of snout (EN/NS 0.97), separated from each other by distance much larger than distance between eye and nostril (NN/EN 1.68); eye directed anterolaterally, hardly protruding, projecting just beyond margins of head in dorsal view, relatively large (ED/HL 0.35), its diameter shorter than snout (ED/SL 0.84); dorsal surface of eye lids slightly above dorsal surface of head in lateral view; interorbital distance much wider than upper eyelid (IO/EW 1.61) and smaller than internarial distance (IO/NN 0.89); tympanum well visible externally, its annulus well defined; upper jaw with dentition; teeth on premaxilla larger than teeth on maxilla; choanae small, rounded, located far anterolaterally at anterolateral margin of roof of mouth, completely covered by maxillary bone, not visible in ventral view; vomer ridges and teeth absent; tongue long and moderately broad, posteriorly expanded, hardly bilobed, free distally for about two-thirds its length; median lingual process absent.

**Table 1.** Morphometrics of the adult male type specimens of *Arthroleptis nyungwensis* sp. nov. (in mm). For abbreviations, see Materials and Methods.

specimen	status	sex	SVL	HL	HW	EYE	TD	SL	EN	NS
ZFMK 104075	holotype	male	16.5	6.1	5.4	2.1	1.0	2.5	1.3	1.4
ZFMK 104076	paratype	male	16.0	5.6	5.4	1.9	0.9	2.2	1.1	1.0
ZFMK 104077	paratype	male	16.1	5.6	5.1	2.0	0.9	2.5	1.2	1.1
specimen	status	NN	EE	EW	IO	HND	3FL	FoL	TFL	THL
ZFMK 104075	holotype	2.2	3.2	1.2	1.9	6.9	5.0	7.1	6.9	7.3
ZFMK 104076	paratype	2.0	3.1	1.0	2.0	6.1	5.5	7.1	7.2	7.4
ZFMK 104077	paratype	1.9	3.0	1.0	2.0	6.8	4.7	7.1	7.3	7.1

Dorsal surfaces of head, trunk, and limbs and lateral surfaces of trunk finely sha-greened (Figure 1A); median skin raphe barely visible on dorsal side in preservative, only discernible on head surface; supratympanic region smooth with no dermal fold; ventral side of head smooth; vocal sac present; chest and anterior part of abdomen smooth, posterior part of abdomen weakly areolate; ventral side of limbs smooth (Figure 1B); short transverse fold above vent.

Forelimbs slender; hand small, but with very long third finger (HND/SVL 0.42); few keratinous spines along lateral sides of third finger; tips of fingers not enlarged into small disc, except slightly in Finger III; relative length of fingers: I < IV < II < III; subarticular tubercles well developed, numbering one on Fingers I and II, three on Finger III and two on Finger IV, proximal tubercles on Fingers III and IV and tubercles on Fingers I and II singular, rounded; distal tubercles on Fingers III and IV indistinctly bipartite; fingers without webbing; thenar tubercle small, low and oval; inner palmar tubercle large, low, and rounded, outer palmar tubercle absent.

Hind limbs slender, moderately long; heel reaching to level of eye when legs adpressed forwardly to body; crus moderately long (TFL/SVL 0.42), slightly shorter than thigh (TFL/THL 0.94); foot length about equal to crus length (FOL/TFL 1.03); relative length of toes: I < II < VI < III < IV; toe tips slightly enlarged into small disks; subarticular tubercles singular, numbering one on Toes I and II, two on Toes III and V, and three on Toe IV; toes without webbing; inner metatarsal tubercle small, rounded and moderately prominent, about one-third length of metatarsus of Toe I; outer metatarsal tubercle indiscernible.



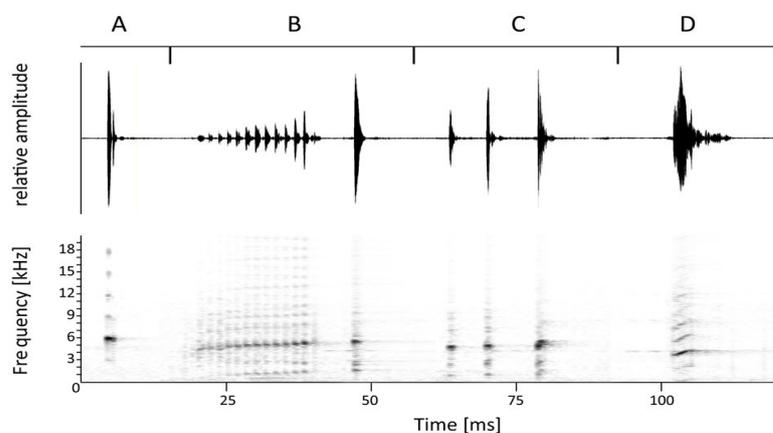
**Figure 1.** (A) Dorsolateral view and (B) ventral view of the holotype (ZFMK 104075) of *Arthroleptis nyungwensis* sp. nov. in life. (C) Dorsolateral view and (D) ventral view of an adult male (JMD 599) of *Arthroleptis nyungwensis* sp. nov. in life. (E) Dorsolateral view and (F) ventral view of one of the subadult paratypes (SMNS 15743) of *Arthroleptis nyungwensis* sp. nov. in life.

**Colouration in life.** Dorsal basic colouration light brown. Slightly darker brown pattern on head and trunk hardly discernible, consisting of dark transversal interorbital bar and faint broad longitudinal band along spine (Figure 1A). Top of head anterior to interorbital bar lighter brown. Dark brown stripe in straight line along ventral part of canthus rostralis from tip of snout to anterior edge of eye, continued downwardly curved from posterior edge of eye almost to arm insertion, just touching the posterodorsal edge of tympanum (Figure 1A). Lateral sides of head and trunk lighter, greyish to yellowish brown; dorsal side of upper arms and hands yellowish brown. Sides of head and forearm loosely, flanks more or less regularly and densely scattered with small white dots (Figure 1A). Base colour of ventral side yellowish brown with tiny dark brown speckles, most densely on ventral side of head; ventral side of head along the jaw and ventral parts of forelimbs darker reddish brown; ventral parts of leg and lateral sides of thigh dark red (Figure 1B); abdomen yellowish brown with two bright yellow longitudinal, slightly concave stripes, faintly merging with each other at posterior end (Figure 1B); small white dots on ventral side, few on forearm and thigh, densely on lateral sides of abdomen, around cloaca and on tarsus; irregularly shaped yellow flecks on abdomen (Figure 1B).

**Colouration in preservative.** Dorsal basic colouration faded to greyish brown. Darker greyish brown dorsal pattern more conspicuous than in life. Ventral colour faded to uniformly yellowish creme-coloured. Dark brown speckling more conspicuous than in life. Yellow colour pattern indiscernible.

**Variation.** The adult male paratypes match the male holotype in general appearance, proportions, colouration, and colour pattern. Mensural variation within the species is shown in Table 1. The dorsal colour pattern was more conspicuous in life in one of the adult males (JMD 599) and in the subadults (Figure 1C,E). In the subadult specimens, the sides of the head and the flanks were dark grey in life and there was a black stripe running in a straight line from the nostril to the anterior edge of the eye and slightly ventrally curved from the posterior edge of the eye above the tympanum to the level of the arm insertion; the venter was light yellow in the centre, darkening to dark yellow then orange on the edges, without the conspicuous yellow stripes (Figure 1E,F).

**Bioacoustics.** Series of advertisement calls of the holotype, two paratypes, another collected and two uncollected males were recorded at different ambient temperatures between 12 °C and 20 °C. Males called from the leaf litter on the forest floor during both night and day. The most-often-emitted vocalisation was a single note (Figure 2A), and I regard this as the advertisement call. The call was repeated in series at irregular intervals of 2.5–8.8 s. In most recordings, the note appeared unpulsed or diffusely pulsatile, but in a few instances, two separate pulses were visible (Figure 2A). The note had a total duration of  $17.4 \pm 6.4$  [11–32] ms ( $N = 5$  males). Individual pulses, when traceable, were separated by an interval of 1 ms duration and had an individual duration of 7 ms (first pulse) and 5 ms (second pulse (Figure 2A)). There was no measurable frequency modulation in shorter notes of less than 20 ms and dominant frequency was at  $5861 \pm 188$  [5531–6029] Hz. A fundamental was present at about 2950 Hz, and harmonics were present at 9000, 12,000, 15,000, and 18,000 Hz (Figure 2A). Prevalent bandwidth was 5400–6300 Hz. In a few longer notes of more than 30 ms, the dominant frequency increased from  $5404 \pm 30$  [5383–5426] Hz in the first third of the note to 5598 Hz in the last third of the note.



**Figure 2.** Waveform (top) and corresponding sound spectrogram of (A) an advertisement call of *Arthroleptis nyungwensis* sp. nov., (B) an aggressive call, consisting of a thirteen-notes group followed by the main note, (C) a variant of the aggressive call, and (D) a possible female courtship call. See text for details.

One of the paratypes (ZFMK 104077) was collected while duetting with another uncollected male. The two males emitted a second call type (Figure 2B,C). It was impossible to differentiate between the two males in the recording, and therefore the following description is based on all recorded calls. The second call type likewise contained a prominent last note lasting  $21.4 \pm 10.1$  [11–39] ms with the dominant frequency increasing from  $5175 \pm 197$  [4866–5469] Hz in the first third of the note to  $5480 \pm 130$  [5167–5727] Hz in the last third of the note (Figure 2B,C). However, the main note was always initiated by a note group that had a much lower amplitude and was separated from the main note by an interval of  $90.1 \pm 13.6$  [71–113] ms. The composition of the note group varied considerably. In about half of the recorded calls, it consisted of an uninterrupted series of 1–13 notes (Figure 2B), in the other half, two or three subgroups, each consisting of 1–3 notes, were separated from each other by intervals of  $37.3 \pm 22.1$  [19–86] ms. As calls with both interrupted and uninterrupted pulse groups were recorded in quick succession, these differences were not attributable to the two different calling individuals. Individual notes within the (sub)groups were repeated at a rate of  $58.6 \pm 4.9$  [47.6–62.5] per second, were weakly pulsatile, and had a duration of  $8.8 \pm 3.3$  [4–14] ms. Amplitude increased from the first to the last note of the group (Figure 2B,C), going along with an increase in dominant frequency from  $4658 \pm 323$  [4177–5124] Hz in the first note to  $4944 \pm 210$  [4608–5254] Hz in the last note (Figure 2B,C). Whereas the interrupted and uninterrupted note groups sounded like a trill, in one case, two widely separated initial notes had a relatively high

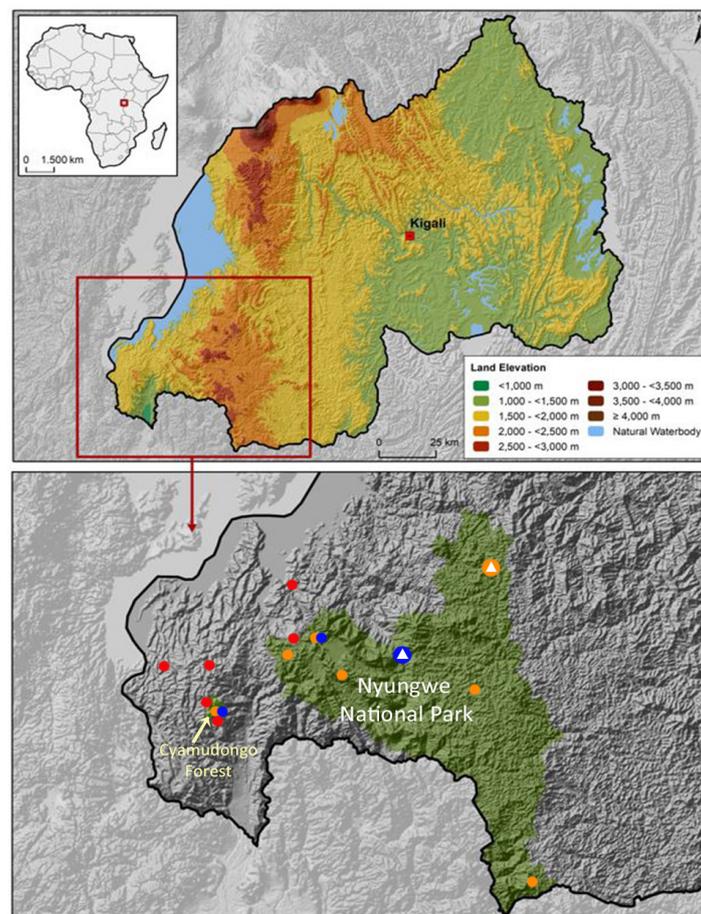
amplitude, giving the impression that the main note was rapidly repeated (Figure 2C). Total duration of the second call type was  $209.1 \pm 40.2$  [139–285] ms.

On the same recording, a third call type was identified. It had been recorded a total of three times and was a brief whistle that consisted of a single pulsatile note, lasting 40, 44 and 74 ms. The dominant frequency was markedly modulated, increasing from  $3760 \pm 108$  [3660–3875] Hz in the first third to  $4205 \pm 25$  [4177–4220] Hz in the last third (Figure 2D).

**Habitat and natural history:** Calling males were observed in moist leaf litter of closed-canopy montane forest between 1800 and 2200 m a.s.l. Subadults were found at the forest edge in Cyamudongo Forest. The species is active at both night and day. The reproduction is unknown, but the species is suspected to deposit eggs in moist leaf litter and to undergo direct development without a tadpole stage.

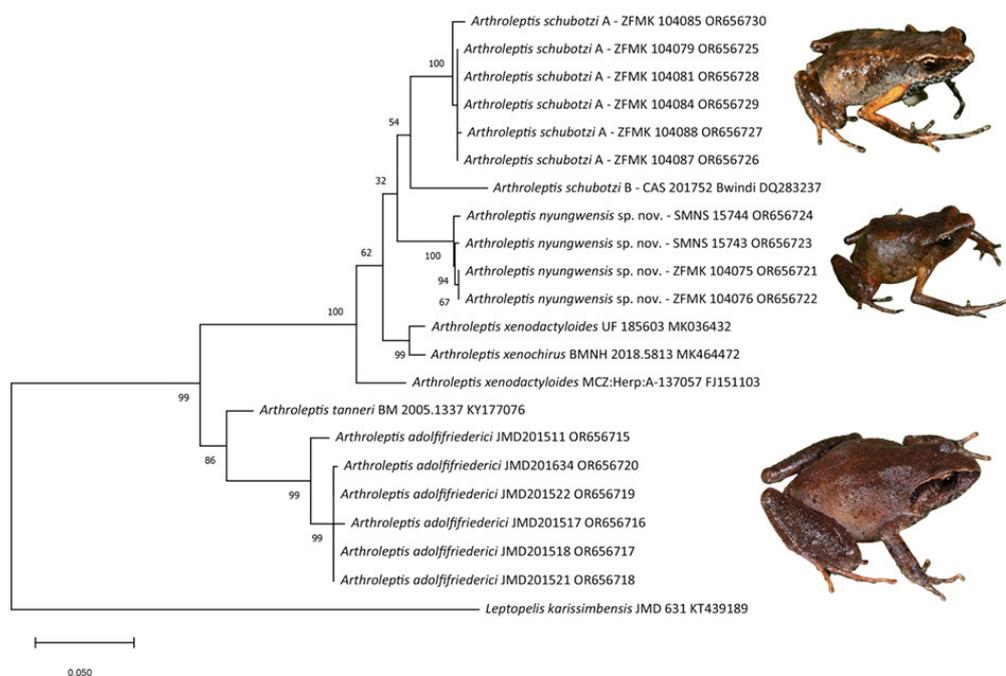
**Etymology:** The species is named after Nyungwe Forest in southern Rwanda.

**Distribution and Conservation:** Despite intensive surveys in Nyungwe Forest and elsewhere in Rwanda [17,29], the species is currently known from only a few localities in the western part of Nyungwe Forest and in Cyamudongo Forest (Figure 3). I propose the species to be classified as “Near Threatened” according to the criteria established by the International Union for Conservation of Nature (IUCN) [7] in both the global Red List and the Rwandan national Red List [17].



**Figure 3.** Map of Rwanda with major elevation zones and natural water bodies (**top**) and detail of map showing Nyungwe National Park including Cyamudongo Forest (**bottom**) in southwestern Rwanda; forested areas are displayed in green. The distribution of the three species of *Arthroleptis* in Nyungwe National Park and surroundings is indicated by coloured dots at localities where specimens were collected or observed: *Arthroleptis adolfriederici* (orange), *A. nyungwensis* sp. nov. (blue), *A. schubotzi* (red). Type localities are marked with a white triangle in the centre of the coloured dot. Map courtesy of S. Seidel.

**Phylogenetic relationships:** The phylogenetic tree resulting from the ML analysis was well supported by nonparametric bootstrap values (Figure 4). All samples of *Arthroleptis nyungwensis* formed a clade that was weakly supported as being sister to a clade containing two deeply divergent clades assigned to *A. schubotzi*, one from Rwanda (*A. schubotzi* A), the other from Bwindi, Uganda (*A. schubotzi* B). The three clades are all from montane forests in the central and northern Albertine Rift. They were sister to a clade containing *A. xenodactyloides* and *A. xenochirus* (Figure 4), two species that are distributed from Angola to southern DRC, southern Tanzania and northwards to Kenya. Another sequence assigned to *Arthroleptis xenodactyloides* was resolved as sister to all aforementioned clades, suggesting that the identification of the source specimens of these three latter sequences need to be revised. The third Rwandan species, *A. adolfriederici*, is only distantly related to *A. nyungwensis* and *A. schubotzi* and appears to be most closely related to *A. tanneri* from the Usambara Mountains in Tanzania (Figure 4). The samples of *A. nyungwensis* differed from each other by 0.00–0.54% and from samples of all other species by at least 4.6% in uncorrected p-distance. Samples of *A. schubotzi* A from Rwanda differed from the sample of *A. schubotzi* B from Uganda by 5.6%.



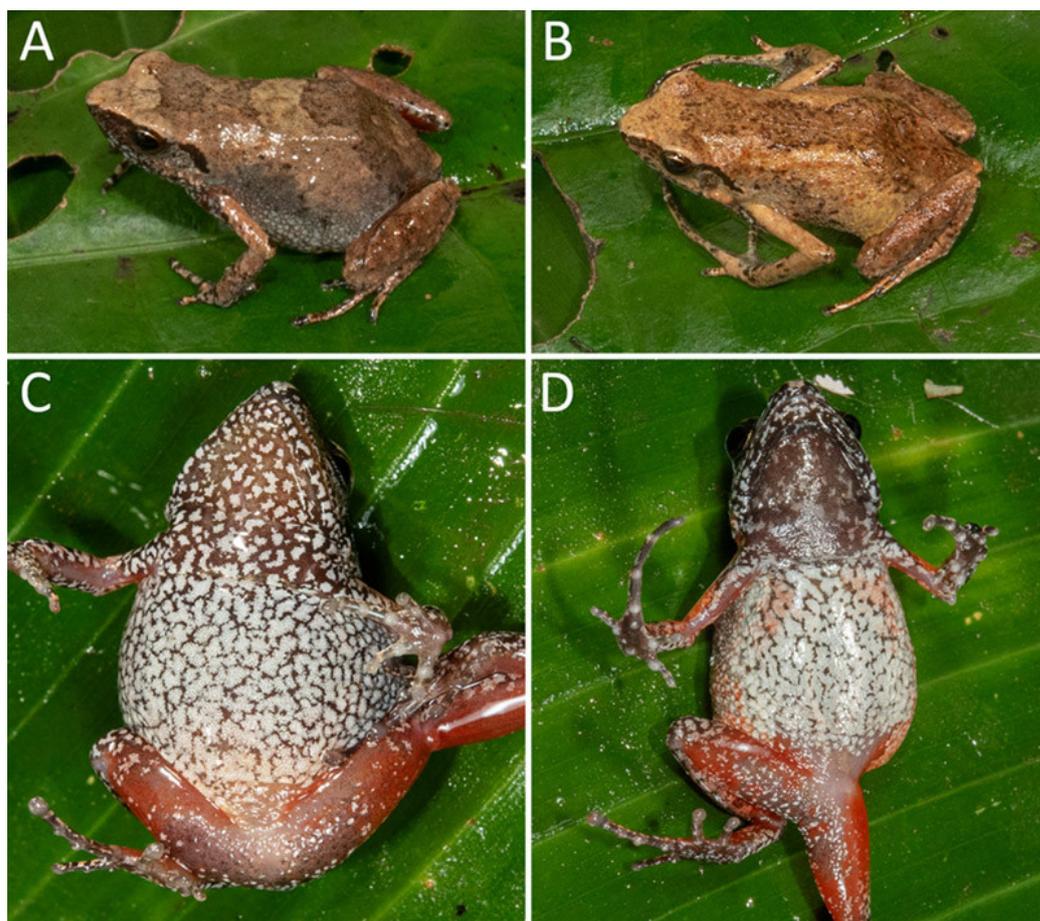
**Figure 4.** Phylogram inferred from mitochondrial nucleotide sequence data of 16S rRNA (563 BP). Numbers below branches are non-parametric bootstrap support values from ML (1000 replicates). Sequences are listed with species name, voucher number, and GenBank accession number, respectively. Specimens of the three species occurring in Rwanda are depicted on the right at the level of their corresponding samples.

**Morphological comparison:** The very small size (SVL of adult males 16.0–16.5 mm) distinguishes *A. nyungwensis* sp. nov. from the other species in the wider region that are larger, i.e., *A. adolfriederici*: males 27.6–32.0 mm; *A. francei* Loveridge, 1953: male 32 mm; *A. phrynoides* (Laurent, 1976): male 20.4 mm; *A. reichei* Nieden, 1911: males to 30 mm; *A. schubotzi*: males 19.9–21.2 mm (including *Schoutedenella discodactyla* Laurent, 1954 with males 17.5–19 mm); *A. spinalis* Boulenger, 1919: male 21.4 mm; *A. stenodactylus* Pfeffer, 1893: males to 33 mm; *A. xenochirus* Boulenger, 1905 (including *Schoutedenella globosa* de Witte, 1921 as well as *A. lameeri* de Witte, 1921 and *Schoutedenella muta* de Witte, 1933; see also [5]): males with enlarged third finger 16.5–24 mm; *A. xenodactylus* Boulenger, 1909: males to 26 mm; *A. xenodactyloides* Hewitt, 1933: males to 25 mm. The very small inner metatarsal tubercle distinguishes the new species from all remaining species with large,

elongate, prominent inner metatarsal tubercle that is about two-thirds the length of the first metatarsus, i.e., *A. loveridgei* de Witte, 1933 (also outer palmar tubercle present, ventral side of thigh coarsely granular, discs of toes strongly enlarged vs. outer palmar tubercle absent, thigh smooth, discs of toes slightly enlarged); *A. sylvaticus* (Laurent, 1954) (also discs of toes strongly enlarged vs. slightly enlarged); and *A. xenochirus* Boulenger, 1905 (also venter uniformly off-white, throat grey in males vs. venter reddish to orange with longitudinal yellow stripes and white dots, throat yellowish brown with tiny dark brown speckles). *Arthroleptis fichika* Blackburn, 2009 and *A. kidogo* Blackburn, 2009 are known only from females that are much smaller (SVL 13.5–14.2 mm and 13.9–14.1 mm, respectively) than the new species, and differ in having a dappled ventral colour pattern [7].

Morphological differentiation between the similarly sized species of *Arthroleptis* from the Albertine Rift is hampered by the shortage of external diagnostic characters, especially in preserved specimens, in combination with the briefness of the original descriptions that specify only a handful of characteristics, some of which are not diagnostic. In addition, the whereabouts and the identity of the types of several species described by Laurent [3,16] are currently vague. The life colouration can be a useful character to easily distinguish between species if it is thoroughly documented. The distributional range of *Arthroleptis nyungwensis* sp. nov. overlaps with that of *A. schubotzi* in western Rwanda, although the two species are usually found in different habitats. The Rwandan specimens match the types of *A. schubotzi* and the types of its junior synonym *Schoutedenenella kivuensis* de Witte, 1941, very well. *Arthroleptis schubotzi* (males 19.9–21.2 mm, male syntype of *S. kivuensis* 19.8 mm; females 21.1–21.6 mm; female holotype currently 19.2 mm, previously measured 21 mm [13] and 20 mm [20], female syntype of *S. kivuensis* 20.6 mm) is significantly larger than *A. nyungwensis* sp. nov. (males 16.0–16.5 mm), has relatively shorter legs (TFL/SVL 0.36–0.41 vs. 0.42–0.46 in *A. nyungwensis*), a relatively larger tympanum (TD/EYE in adult males >0.60 vs. <0.48), a relatively shorter head (HL/SVL 0.31–0.33 vs. 0.35–0.37), and its dorsum is granular, covered with small, low tubercles, especially in males (Figure 5; vs. dorsum finely shagreened). It is most easily distinguished from *A. nyungwensis* sp. nov. (venter reddish to orange with longitudinal yellow stripes and white dots, Figure 1) by its conspicuous ventral pattern consisting of an off-white background with dark reticulation, with the ventral side of the head having a higher proportion of dark pattern than the breast and abdomen in females and the gular region of males being black with few whitish flecks, and bright red thighs (Figure 5). This colour pattern was also described in the female holotype of *A. schubotzi* [20] and described and depicted for the types of *Schoutedenenella kivuensis* [15]. There are a number of similarly sized, but poorly defined taxa from eastern DRC that are only known from the type series [3,16] and whose taxonomic status is doubtful. A very similar ventral colour pattern to *A. schubotzi* is present in the types of *Arthroleptis mossoensis* (Laurent, 1954), *Schoutedenenella discodactyla* (currently considered a junior synonym of *A. schubotzi*) and *A. pyrrhoscelis* Laurent, 1952, and these taxa are readily distinguished from *A. nyungwensis* sp. nov.: *Arthroleptis mossoensis* was described from Murugaragara, Mosso, Territoire de Rutana, Burundi, at an altitude of 1200 m, based on a single female (SVL 22.4 mm) that remains the only specimen of the species ever collected. It further differs from *A. nyungwensis* sp. nov. in its short hindlimbs (TFL/SVL 0.36 vs. 0.42–0.46), the tibio-tarsal articulation only reaching the shoulder (vs. reaching the eye). *Arthroleptis pyrrhoscelis* from the Kabobo Plateau in eastern DRC is similar in size (male types 15.1–16.2 mm) to *A. nyungwensis* sp. nov., but has shorter hindlimbs, the tibiotarsal joint reaching only to tympanum (vs. to eye). The posterior part of the abdomen and the ventral side of the thighs are coarsely granular (vs. weakly areolate), an outer palmar tubercle and several prominent metacarpal tubercles are present (vs. absent), and the inner metatarsal tubercle is large, elongate and prominent (vs. small and rounded). *Schoutedenenella discodactyla* from Lutunguru in the North Kivu Province of DRC (currently referred to *A. schubotzi*) differs by its markedly dilated discs of fingers and toes (vs. tip of third finger only and tips of toes slightly enlarged). *Arthroleptis vercammeni* (Laurent, 1954) from Mwana in eastern DRC is a very small species (SVL of males 13–15 mm, of females 15–17 mm [3])

and thus even smaller than *A. nyungwensis* sp. nov. (males 16.0–16.5 mm), the throat is blackish in males (vs. yellowish brown with tiny dark brown speckles), the tibia is noticeably longer than the foot (TFL/FOT 1.10–1.25 vs. 0.97–1.03 in *A. nyungwensis*), and the tips of fingers and toes are clearly dilated [3] (vs. tip of third finger only and tips of toes slightly enlarged). *Arthroleptis hematogaster* (Laurent, 1954) is a poorly known species from the South Kivu Province in eastern DRC. The species is similar in size (only known male 16.5 mm), but is reported to have a uniform ventral colouration varying from orange-red to blood-red [3] (vs. yellowish brown) and lacks the distinct two longitudinal yellow ventral stripes and the white dots on venter, arms and thighs that are present in *A. nyungwensis* sp. nov., the foot is relatively shorter with FOL/SVL 0.38–0.41 (vs. 0.43–0.45), and finger tips and toe tips are strongly enlarged (vs. slightly enlarged).



**Figure 5.** *Arthroleptis schubotzi* from Cyamudongo Forest, Rwanda, in life. (A) Dorsal view of adult female (ZFMK 104089) and (B) adult male (ZFMK 104090). (C) Corresponding ventral view of adult female and (D) adult male, showing the distinct ventral colour pattern.

**Bioacoustic comparison:** The advertisement calls of only about half the species of *Arthroleptis* are known [1]. The advertisement call of *Arthroleptis nyungwensis* sp. nov. (single note,  $17.4 \pm 6.4$  [11–32] ms duration, dominant frequency at  $5861 \pm 188$  [5531–6029] Hz; Figure 2) differs from all described calls of its congeners. The call of one of the two other species of *Arthroleptis* recorded from Rwanda, *A. adolfriederici*, is similar in consisting of a single, brief (52–65 ms) note, but has a much lower dominant frequency of about 2900 Hz [17]. The known advertisement calls of the phylogenetically closely related species differ markedly from the call of *A. nyungwensis* sp. nov., i.e., *A. xenochirus*: high-pitched trill, seven notes, repeated at a rate of 15/s, dominant frequency at 4600 Hz [22]; *A. xenodactylus*: brief whistle, duration 50 ms, repeated two or three times per second, dominant frequency at 6400–7000 Hz [22]; *A. xenodactyloides*: single note, 88–95 ms, repeated at an interval

of 340–390 ms, composed of three pulses, each lasting 16–24 ms, dominant frequency at 6300–6500 Hz [30]; brief, cricket-like chirp, consisting of three brief clicks, dominant frequency at 5500 Hz [22]; call duration 0.05–0.1 s, repeated every 0.3–0.7 s, dominant frequency 5.2–6.4 kHz, 2–4 pulses [31].

Based on observations of males of its junior synonym *Schoutedenella kivuensis*, the call of *A. schubotzi* has been described onomatopoeically as “cri-cri, cri-cri” and characterized as “extremely piercing and very characteristic cry, absolutely similar to that of *S. globosa*” [= *A. xenochirus*], “quite comparable to the ‘song’ of our cricket, but much louder” [15]. This was subsequently cited as a “harsh series of double chirps” [1,22]. The call description agrees with the results of the analysis of call recordings of *A. schubotzi* from Rwanda [17]: call duration 246–429 ms, 5–6 notes, each lasting 23–29 ms and composed of three pulses, internote interval 34–40 ms, dominant frequency 4331–4450 Hz. The advertisement call of males assigned to *A. schubotzi* from Kibale National Park, Uganda, differs from the advertisement call of Rwandan specimens in consisting of only either two or three notes, the first two being separated from each other by an interval of 80 [59–98] ms, the second and third by an interval of 94 [84–113] ms; in having a slightly higher dominant frequency of 4661 [4462–4892] Hz; and in a briefer call duration of 158 [92–273] ms [32]. The advertisement calls of both populations assigned to *A. schubotzi* differ from the advertisement call of *A. nyungwensis*.

#### 4. Discussion

The mountains of the Alberine Rift harbour the type localities for many small *Arthroleptis* species [3,8,13–16]. Several of these species are known only from their types, and in some cases their taxonomic status is unclear. Examination of the available type material and a survey of the available literature suggest that the group is in urgent need of a thorough revision. For example, *Arthroleptis mossoensis* and *A. pyrrhoscelis* match *A. schubotzi* in many characteristics such as size, relative leg length, rough dorsal skin, and the conspicuous ventral colour pattern, and were described from roughly the same geographic region. The status of these taxa currently recognized as distinct species need to be reassessed. On the other hand, the ML analysis of phylogenetic relationships revealed two deeply divergent clades between populations currently assigned to *A. schubotzi*, one from Rwanda (“*A. schubotzi* A”), the other from Bwindi, Uganda (“*A. schubotzi* B”). There are also marked advertisement-call differences between populations of *A. schubotzi* from Rwanda [17] and from Kibale, Uganda [32]. These differences indicate that two or more unrecognized species could be admixed under the nominal taxon *A. schubotzi*. These taxonomic issues will be addressed in an upcoming study.

*Arthroleptis nyungwensis* is usually found in closed-canopy montane forest and rarely at the forest edge, whereas *A. schubotzi* is abundant at forest edges and in anthropogenically disturbed habitats. Therefore, both species usually occur in parapatry to each other in western Rwanda. Calling males of both species were, however, found syntopically at one locality in Cyamudongo Forest, in a transition between secondary forest and a buffer zone. The advertisement call of *A. nyungwensis* differs more markedly from the advertisement call of *A. schubotzi* from Rwanda than from the allopatric population in Kibale, Uganda, assigned to *A. schubotzi* [32]. The second call type recorded from one of the paratypes of *A. nyungwensis* is very similar to the vocalisation of *A. schubotzi* from Kibale National Park, Uganda, which was identified as an aggressive call (sensu [33]) and consisted of either one or two elongated pulse trains, followed by a single high-pitched metallic note similar to those of the advertisement call [32]. Given the circumstances at the time of the recording and its similarity to the aggressive call of “*A. schubotzi*”, the second call type of *A. nyungwensis* appears to be an aggressive call directed towards competing males during male-male interactions. The third call type of *A. nyungwensis* differs markedly from the advertisement and the aggressive call of males. Considering its characteristics and the fact that the dominant frequency was much lower than in calls emitted by males, the most plausible explanation is that it was the courtship call of a female [33] that had also been

present at the scene but had been overlooked. This, however, needs to be confirmed by a direct observation of a calling female.

Several new species of amphibians and reptiles have recently been discovered or recorded for the first time from Nyungwe Forest and its surroundings [17,34–38]. The discovery of yet another new species from Nyungwe Forest and Cyamudongo Forest indicates that the herpetofaunal diversity of these forests is still not fully assessed. Future field surveys will probably yield additional records, especially in the vast unexplored areas that still remain in Nyungwe Forest. The discovery of another amphibian species that is locally endemic to Nyungwe National Park underlines the importance of the national park for the protection of the area's biodiversity.

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## Appendix A

**Table A1.** Specimens of *Arthroleptis* species examined for this study, their taxonomic status, voucher number, present-day country of origin and collection locality.

Species	Status	Voucher #	Country of Origin	Locality
<i>A. adolfriederici</i>	syntype	ZMB 21787	Rwanda	Rugegewald [=northeastern Nyungwe Forest]
<i>A. adolfriederici</i>	topotype	201511	Rwanda	Rugegewald [=northeastern Nyungwe Forest]
<i>A. adolfriederici</i>	-	JMD 597, 202103	Rwanda	Nyungwe Forest
<i>A. adolfriederici</i>	-	JMD 201517–23	Rwanda	Gishwati Forest
<i>A. adolfriederici</i>	-	JMD 201634	Rwanda	Cyamudongo Forest
<i>A. hematogaster</i>	possible types	RMCA "Caja 40, Lote 60" (2 specs.)	DRC	May ya Moto
<i>A. loveridgei</i>	syntypes	RMCA 2624–25	DRC	Arebi
<i>A. nyungwensis</i>	holotype	ZFMK 104075	Rwanda	Rukuzi (2.4635 S, 29.2293 E; 2200 m), Nyungwe National Park

Table A1. Cont.

Species	Status	Voucher #	Country of Origin	Locality
<i>A. nyungwensis</i>	paratype	ZFMK 104076	Rwanda	Rukuzi (2.4635 S, 29.2293 E; 2200 m), Nyungwe National Park
<i>A. nyungwensis</i>	paratype	ZFMK 104077	Rwanda	Cyamudongo Forest (2.546 S, 28.989 E, about 2000 m)
<i>A. nyungwensis</i>	paratype	SMNS 15743–44	Rwanda	Cyamudongo Forest (2.5378 S, 29.9942 E; 1830 m)
<i>A. phrynoides</i>	holotype	RMCA 75-43-B-23	DRC	Lomami, Terr. de Lomela, Sankuru
<i>A. pyrrhoscelis</i>	possible types	RMCA “Caja 9, Lote 98” (5 females, 2 males)	DRC	Haute Lubitshako, Terr. de Fizi (Kabobo)
<i>A. pyrrhoscelis</i>	possible types	RMCA “Caja 39, Lote 64” (many specs.)	DRC	Haute Lubitshako, Terr. de Fizi (Kabobo)
<i>A. schubotzi</i>	holotype	ZMB 21774	Burundi	Usumbura [=Bujumbura]
<i>A. schubotzi</i>	-	ZMB 22860	DRC	Insel Kwidschwi
<i>A. schubotzi</i>	-	ZFMK 104078–104092, JMD 201909–10	Rwanda	Cyamudongo Forest
<i>A. schubotzi</i>	-	ZFMK 104081,-84,-85	Rwanda	Buhinga
<i>A. schubotzi</i>	syntypes of <i>Schoutedenella kivuensis</i>	RMCA 48.783–784	DRC	N’Zulu (Lac Kivu)—Passe de Sake (alt. 1500 m.)
<i>A. schubotzi</i>	possible types of <i>Schoutedenella discodactyla</i>	RMCA “Caja 31, Lote 81” (2 specs.)	DRC	Lutunguru
<i>A. spinalis</i>	holotype	RMCA 455	DRC	Plaine St. Louis (Baudounville)
<i>A. spinalis</i>	holotype of <i>A. boulegeri</i>	RMCA 534	DRC	Plaine St. Louis (Baudounville)
<i>A. vercammeni</i>	-	RMCA “Caja 4, Lote 3”	DRC	Mwana
<i>A. xenochirus</i>	syntypes of <i>Arthroleptis globosa</i>	RMCA 586, B 585 / A–F (7 specs.)	DRC	Lofoi
<i>A. xenochirus</i>	syntypes of <i>Arthroleptis lameeri</i>	RMCA 180–180D	DRC	Lofoi
<i>A. xenochirus</i>	syntypes of <i>Schoutedenella muta</i>	RMCA 16219–16248	DRC	Kando près Tenke

**Table A2.** Samples of species used for molecular genetic analyses, their geographic origins, voucher specimens, GenBank accession numbers, and original sources.

Species	Location	Voucher	GenBank#	Original Source
<i>Arthroleptis adolfriederici</i>	Nyungwe Forest, Rwanda	JMD 201511	OR656715	this study
<i>A. adolfriederici</i>	Gishwati Forest, Rwanda	JMD 201517	OR656716	this study
		JMD 201518	OR656717	
		JMD 201521	OR656718	
		JMD 201522	OR656719	
<i>A. adolfriederici</i>	Cyamudongo Forest, Rwanda	JMD 201634	OR656720	this study
<i>A. nyungwensis</i>	Rukuzi, Nyungwe National Park, Western Province, Rwanda	ZFMK 104075	OR656721	this study
		ZFMK 104076	OR656722	
<i>A. nyungwensis</i>	Cyamudongo Forest (2.5378 S, 29.9942 E; 1830 m), Western Province, Rwanda	SMNS 15743	OR656723	this study
		SMNS 15744	OR656724	
<i>A. schubotzi</i>	Cyamudongo Forest, Rwanda	ZFMK 104079	OR656725	this study
		ZFMK 104087	OR656726	
		ZFMK 104088	OR656727	
<i>A. schubotzi</i>	Buhinga, Rwanda	ZFMK 104081	OR656728	this study
		ZFMK 104084	OR656729	
		ZFMK 104085	OR656730	
<i>A. schubotzi</i>	Bwindi Impenetrable National Park, Uganda	CAS 201752	DQ283237	[12]
<i>A. xenochirus</i>	Lukwakwa, Zambia	BMNH 2018.5813	MK464472	[39]
<i>A. xenodactyloides</i>	Angola	UF185602	MK036432	[40]
<i>A. xenodactyloides</i>	Misuku Mountains, Mughese Forest Reserve, Malawi	MCZ:Herp:A-137057	FJ151103	[6]
<i>Leptopelis karissimbensis</i>	Rwasenkoko swamp, Nyungwe National Park, Rwanda	JMD 631	KT439189	[41]

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