Taxonomy, systematics, and ecology of selected amphibian taxa from Rwanda

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Hiermit erkläre ich, dass der Eigenanteil an den folgenden Publikationen inhaltlich und prozentual wie folgt ist:

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Eigenanteil (ca. 40 %) besteht im Messen eines Teils der Daten, ihrer Analyse und Beiträgen zur Diskussion.

Chapter III: Dehling J. M. & Sinsch U. (2013) Diversity of Ridged Frogs (*Ptychadena* spp.) in wetlands of the upper Nile in Rwanda: Morphological, bioacoustic, and molecular evidence. – Zoologischer Anzeiger - A Journal of Comparative Zoology 253: 143–157.
Eigenanteil (ca. 90 %) besteht im Messen aller Daten, ihrer Analyse und der Konzeption und dem Schreiben der Publikation.

Chapter IV: Dehling J. M. & Sinsch U. (2013) Diversity of *Ptychadena* in Rwanda and taxonomic status of *P. chrysogaster* Laurent, 1954 (Amphibia, Anura, Ptychadenidae). – ZooKeys 356: 69–102.
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I

Introduction

About one-third (32 percent) of the world's amphibian species are known to be threatened by extinction in the wild, and the threat status of another 25 percent is unknown due to insufficient data (Stuart *et al.* 2008). At the same time, amphibians have the highest rate of new species discoveries among vertebrates with between 148 and 273 species having been described as new or elevated from synonymy each year for the last decade (Amphibiaweb 2014). There is no sign that this rate is decreasing which indicates that there is a high number of amphibian species still unknown or unrecognized by science, many of which could be threatened or even become extinct before being discovered. Therefore, basic biodiversity research on this group is urgently needed. Taxonomy and systematics are classic biological disciplines, dating back as far as Aristotle (384-322 BC; Storch *et al.* 2007). Unfortunately, they are therefore increasingly regarded as "old" or "out-of-date" sciences. However, taxonomy still is the base of all biological research (e.g. Wägele *et al.* 2011). Results of biological studies are futile if they cannot be attributed to a certain taxonomic unit (usually a species), and findings of a study can be distorted if they have derived unknowingly from several, not distinguished species instead of a single one. Furthermore, studies on the diversity within certain taxa and their relationships to each other can serve as more than mere species inventories but can help to elucidate or corroborate biogeographic patterns, past geological events, or evolutionary processes; examples for which include several studies on amphibians (e.g. Biju & Bossuyt 2003; Vences *et al.* 2004; Measey *et al.* 2007; Blackburn 2009; Veith *et al.* 2009; Loader *et al.* 2014). Well-established taxonomy is also the base for efficient conservation biology, because unresolved taxonomy can easily result in the negligence or the underestimation of a species' threat status. Habitat loss mostly due to conversion of natural habitats to farmland has been identified as the greatest threat to amphibians (Stuart *et al.* 2008). Therefore, basic taxonomic research should primarily focus on groups with largely unresolved taxonomy in geographical regions that have not yet been

sufficiently explored by scientists, have a high human population density, and are suspected to hold a high number of threatened species. The Central African Albertine Rift has been identified as one of seven regions in Africa with major concentration of threatened amphibian species and with a high population density (Andreone *et al.* 2008, Katariya & Chanson 2008). These concentrations of threatened species correlate with those for other taxa (Collar & Stuart 1988; Baillie *et al.* 2004). At the same time, the region's herpetofauna has not received much attention by scientists during the last 60 years and its amphibian species richness is expected to be higher than the current data indicate (Andreone *et al.* 2008). In this thesis I investigate the taxonomy, systematics, and ecology of selected amphibian groups in the Albertine Rift, with a regional focus on Rwanda.

The Central African Albertine Rift ranges approximately from Lake Albert in the north to the southern tip of Lake Tanganyika (Plumptre *et al.* 2007; Figure 1). It is considered the western branch of the East African Rift system and its formation probably began 25 million years ago (Roberts *et al.* 2012). Several mountain ranges are situated at the edges of the rift, including the Lendu Plateau and the Rwenzori Mountains in the north; the central part of the Mitumba Mountains with the highest peaks Kahuzi and Biega and the Itombwe and Kabobo Plateaus in the central west; the Virunga Mountains, the Nyungwe/Kibira Forest, and the Bururi Highlands in the central east, and the Marungu Highlands, the Kungwe-Mahale Mountains, and the Mbisi Mountains in the south (Figure 1). The mountains and highland plateaus of the region have been identified as probable forest refuge areas in which forest-dwelling species outlasted fluctuating periods of much drier climate during the Pleistocene (e.g. Diamond &Hamilton 1980; Mayr & O'Hara 1986; Fjeldså & Lovett 1997; Linder 2001; Roy *et al.* 2001, Anthony *et al.*, 2007) and the region is considered a hotspot of plant and vertebrate diversity and harbours more species and more endemic vertebrates than any other region in continental Africa (Myers *et al.* 2000; Brooks *et al.* 2001; Plumptre *et al.* 2007). About 20 percent of the African amphibian species occur in the Albertine Rift (Plumptre *et al.* 2007), and one-third of these are endemic to the area. Despite the high diversity, relatively little attention has been paid to the amphibians of the Albertine Rift during the last decades and most of the knowledge about them stems from explorations during the first half of the 20th century.

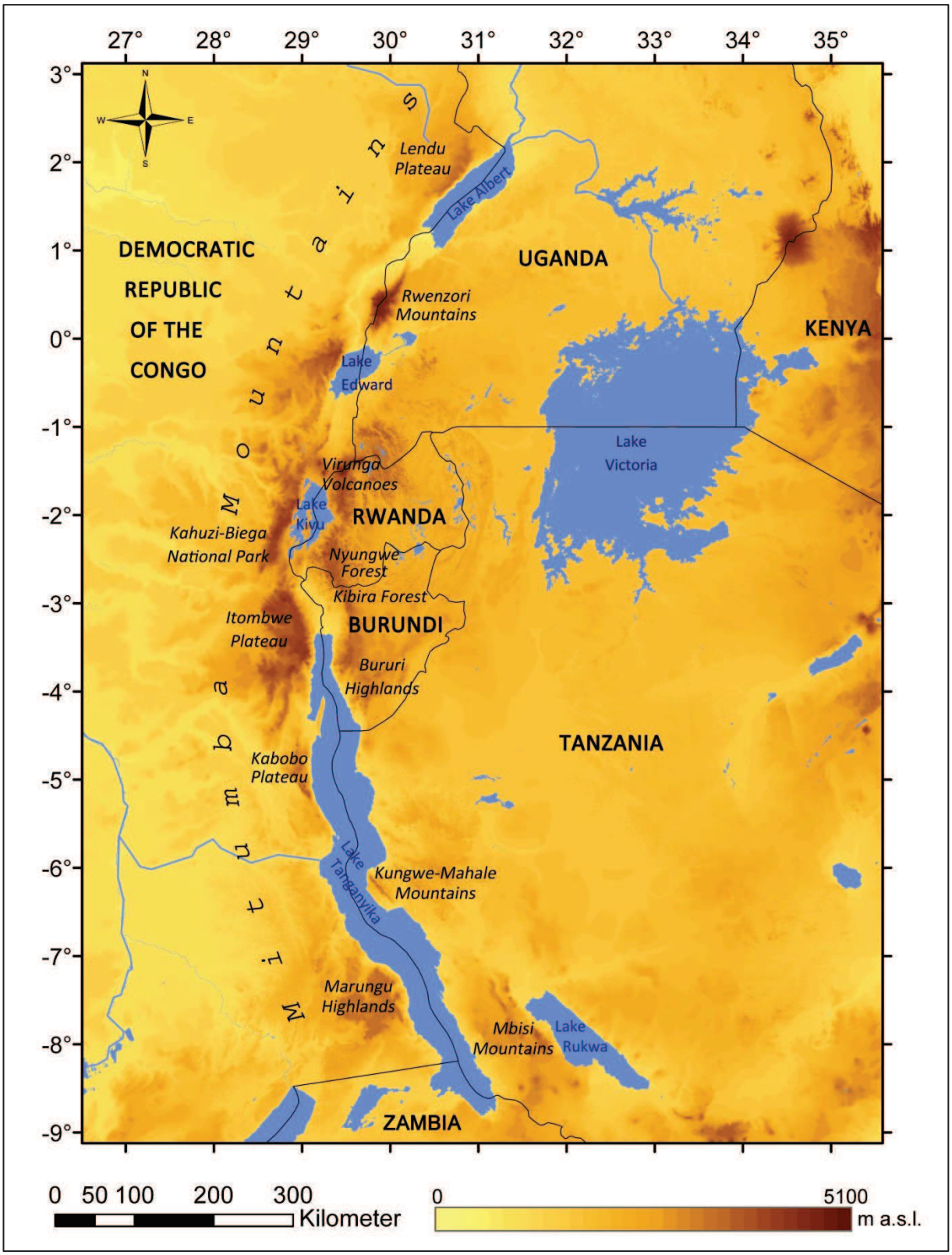

Figure 1. Map of the Central African Albertine Rift showing the major mountain ranges.

The Central African country Rwanda is situated between 1°04' and 2°51' S and 28°53' and 30°53' E (Figure 1). Lake Kivu and the valley of the Ruzizi River in the west of the country are part of the Albertine Rift. The eastern edge of the rift, which in Rwanda runs from the Virunga Volcanoes in the northwest to the Nyungwe Forest in the southwest, divides the westernmost part of Rwanda from the central plateau and the eastern lowland and also represents the watershed between the Congo River and the Nile River (Figure 1). Elevations within Rwanda range from about 925 m a.s.l. in the southwestern Bugarama plains to the top of the volcano Karisimbi at 4507 m a.s.l. The altitudinal gradient in combination with the country's topography favours the presence of a wide range of habitat types (Fischer & Hinkel 1992). Furthermore, Rwanda is not only part of the endemic-rich Albertine Rift region but also situated at the edges of two further major biogeographical regions, the Central African rainforests and the East African savanna (Burgess *et al.* 2004; Blackburn *et al.* 2008; Zimkus *et al.* 2010), allowing species that have originated in either of the three regions to occur within the boundaries of the country. However, the diversity of the Rwandan amphibians and their distribution within the country are far from being fully assessed. In regional treatments of amphibians, Rwanda is usually either not covered (Schiøtz 1975; Channing & Howell 2006) or the presence and distribution of species in Rwanda is extrapolated from data from outside the country (e.g. Branch 2005; Spawls *et al.* 2006, IUCN 2013, Frost 2014) or coarsely described based on information from the middle of the 20th century (Schiøtz 1999, Frost 2014). Despite the fact that herpetological field work has been conducted for more than a century, the country must be considered poorly explored in comparison to other countries of the region like Tanzania, Kenya and Uganda.

The first major collection of Rwandan amphibians was made by Hermann Schubotz on the scientific expedition under Duke Adolf Friedrich of Mecklenburg from 1907 to 1908 (Schubotz 1913). Additional specimens were collected after the expedition by the Austrian zoologist Rudolf Grauer in the same region, and the combined material, which had been deposited in the collection of the Zoologisches Museum Berlin (ZMB), was subsequently examined by Fritz Nieden who identified 20 species of frogs among the 400 collected specimens, of which he described five as new (Nieden 1911, 1913). Species of the genus "*Rappia*" (to which Nieden at that time referred specimens of the genera *Afrixalus*, *Hyperolius*, and *Leptopelis*) were not treated by Nieden "because of the associated

difficulties" and Nieden (1913) stated that "additional research is required to get to know entirely the fauna of this region". The Rwandan frogs including those of the genus "*Rappia*" were re-examined by Ernst Ahl, who during his engagement as "Wissenschaftlicher Oberassistent" at the Department of Herpetology and Ichthyology of the ZMB published a series of papers, in which he described many new species (e.g. Ahl 1924, 1925, 1929, 1930, 1931a, 1931b). After the territory which is now Rwanda had become part of the Belgian Congo after the First World War, expeditions to explore the Rwandan fauna were primarily conducted by Belgian zoologists, but also by researchers from other countries. Noteworthy is the expedition of the Harvard Medical School in 1926 during which amphibians, including the types of a new frog species, were collected in Rwanda (Barbour & Loveridge 1929). The most substantial contribution to the exploration of the Rwandan herpetofauna so far was made by Belgian herpetologists, most notably Gaston-François de Witte and Raymond Ferdinand [Louis-Philippe] Laurent. Several expeditions to the Belgian Congo were lead and accompanied by de Witte during which he made important collections of amphibians and reptiles (e.g. de Witte 1933, 1934, 1941, 1953, 1966). Laurent was arguably the most important contributor. Starting in 1940 he wrote more than 100 publications on Africa's herpetofauna including Rwandan amphibians (e.g. Laurent 1940, 1941, 1943, 1947, 1950a, 1950b, 1951, 1952, 1954, 1956a, 1956b, 1957, 1961, 1972a, 1972b, 1973, 1976, 1983a, 1983b). Whereas his earlier work was based on collections in the Royal Museum for Central Africa in Tervuren, Belgium and other European museums and on loans he received from Parcs Nationaux du Congo Belge, he also made huge own collections on expeditions in Central Africa between 1950 and 1960 (Stewart & Halloy 2002). Laurent provided countless new locality records of amphibians and described a huge number of new taxa. Starting in the mid-1980s, Harald Hinkel from the University of Mainz has been investigating the Rwandan herpetofauna (e.g. Fischer & Hinkel 1992; Hinkel 1993, 1996). Apart from his own publications, his data on reptiles have been incorporated in regional field guides (Spawls *et al.* 2004, 2006). He also described and rediscovered (together with John Measey, Eberhard Fischer and Bonny Dumbo) the only caecilian species which is known from the Albertine Rift, *Boulengerula fischeri* (Nussbaum & Hinkel 1994; Measey *et al.* 2011). In recent years, the Rwandan herpetofauna has been explored by several groups from the USA (University of Texas at Arlington), Italy (Museo Tridentino di Scienze Naturali, Trento), and Germany (Zoologisches Forschungsmuseum Alexander Koenig, Bonn, and University of Koblenz).

Published results of these studies include a tadpole description (Roelke *et al.* 2009), an extension of the range of *Leptopelis karissimbensis* (Hölting *et al*. 2009), a herpetofaunal inventory of the Volcano National Park (Roelke & Smith 2010), an assessment of montane anuran communities (Sinsch *et al.* 2011), a taxonomic study of two species of *Leptopelis* (Roelke *et al*. 2011), and a paper on the taxonomy of *Hyperolius castaneus* and *H. constellatus* (Greenbaum *et al.* 2013). The results of the fieldwork of the Trento Natural History Museum remain largely unpublished (Menegon 2008; Pupin *et al.* 2013) except for a paper on the systematics of the endemic *Boulengerula fischeri* (Gower *et al*. 2011). Furthermore, some recent phylogenetic studies which comprised species from the Albertine Rift used samples obtained in Rwanda (*Xenopus*: Evans *et al.*, 2004, 2008, 2011; *Phrynobatrachus*: Zimkus & Schick 2010, Zimkus *et al.* 2010).

Currently, about 40 amphibian species have been reported for Rwanda (summarized by Frost 2014) but considering the limited field work carried out so far, the country is expected to be richer in species than current data indicate (Andreone *et al.* 2008), and several additional amphibian species have already been recorded (unpubl. data of H. Hinkel, E. Fischer, U. Sinsch, J. M. Dehling). About 20 percent of the recorded Rwandan amphibians are considered threatened under the criteria of the IUCN, rendering Rwanda one of the African countries with the highest percentage of affected species (Andreone *et al.* 2008). However, this number is not based on detailed studies on population densities within Rwanda but on the overall distribution of these species and their presence in protected areas (IUCN 2013). The actual status of the different amphibian species in Rwanda is barely known and most of the data on the diversity and distribution of the Rwandan amphibians stems from the 1950s. At that time, Rwanda had a population of about 2 million people (United Nations, Population Division 2011). This number has grown to 12 million in 2013, and Rwanda is now the most densely populated country in continental Africa with about 474 inhabitants per sq km (The World Fact Book 2013/2014). The rapid increase in population has generated a growing demand for subsistence agriculture areas, livestock grazing areas, and fuel wood and charcoal, which has put the remaining Rwandan forests under an increasing pressure in terms of encroachments and deforestation (REMA 2009). The area covered by forest in Rwanda has decreased from an estimated 659,000 ha (28 % of the country's dry surface) in 1960 to 240,746 ha (10 %) in 2007 (REMA 2009). More than 80 per cent of the country's

total land surface is exploited for agriculture (ROR 2008, REMA 2009). The major threat to the Rwandan biodiversity including the amphibians is therefore habitat loss due to expanding conversion of natural habitats to farmland. It has not been investigated if habitat loss has had an effect on Rwandan amphibian populations and if and which species can cope with habitat alteration. Therefore, herpetological work is urgently needed to be conducted in Rwanda to assess the current diversity and distribution of species and provide a basis for conservation efforts. The aim of this thesis is to contribute towards a comprehensive inventory of the Rwandan amphibians by applying modern herpetological methods to assess the composition of amphibian communities and the diversity within two taxonomically challenging groups of frogs, the Ridged Frogs of the genus *Ptychadena* and the Reed Frogs of the genus *Hyperolius*. Both genera contain a huge number of species (51 and 130, respectively; Frost 2014), of which in both cases many are notoriously difficult to distinguish. The diversity within both genera in the Albertine Rift is very difficult to assess because species from the area were often described several times under different names, of which most are currently considered synonyms, but were at the same time poorly diagnosed in the original descriptions, and therefore subsequently often confused with each other (e.g. Ahl 1931a, 1931b; Laurent 1940, 1943, 1947, 1950a, 1950b, 1954). Species delimitations in this thesis follow an integrative approach, i.e. combining morphological, bioacoustic, and molecular (DNA barcoding) data. The results are presented in seven separate chapters, all of which contain a specific introduction, an account on materials and methods, results and a discussion, as well as a specific acknowledgement section. All chapters have been published individually in peer-reviewed journals; details on which are provided on the first page of each chapter.

II

Acoustic niche partitioning in an anuran community inhabiting an Afromontane wetland (Butare, Rwanda)

This chapter has been published as:

Ulrich Sinsch[1], Katrin Lümkemann[1], Katharina Rosar[1], Christiane Schwarz[1] & J. Maximilian Dehling[1] (2012) Acoustic niche partitioning in an anuran community inhabiting an afromontane wetland (Butare, Rwanda). – African Zoology 47 (1): 60–73.

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Abstract

The species richness and calling activity of an anuran community inhabiting an agricultural wetland area at 1645 m a.s.l. near Butare, Rwanda, was assessed using visual and acoustic transects. The community included 15 species which were readily distinguishable using morphological, bioacoustic and molecular features. Eight species (*Xenopus victorianus*, *Amietophrynus regularis*, *Ptychadena anchietae*, *P. porosissima*, *Kassina senegalensis*, *Afrixalus quadrivittatus*, *Hyperolius kivuensis*, *H. lateralis*) were taxonomically identified. The remaining seven species (three species of *Hyperolius*, two *Phrynobatrachus*, one *Amietia*, one *Ptychadena*) represent undescribed or currently unrecognized taxa, suggesting a significant magnitude of overlooked amphibian diversity in Afromontane communities. Acoustic niche analysis of the 14 species producing airborne advertisement calls integrated the spatial dimension, i.e. the microhabitat used for calling, the temporal dimension, i.e. the time of day when calling takes place, and the call structure dimension, i.e. the physical features of the advertisement call. Average standardized acoustic niche breadth was narrow

(measured: 0.08, predicted: 0.07) and showed low variability (0.04–0.16) among species, which means that empirical data are in full agreement with the predictions of stochastic niche theory for species-saturated communities. Niche segregation was mainly based on advertisement call features, whereas spatial and temporal niche dimension contributed less. Measured average niche overlap (0.30) was intermediate between random overlap (0.51) and minimum possible overlap (0.11), indicating significant acoustic resource partitioning. The only taxon group with widely overlapping acoustic niches were *Ptychadena* spp., which might indicate a recent invasion of the community by one or two of the three species.

Key words: advertisement call, stochastic niche theory, species saturation, cryptic diversity.

Introduction

Niche theory predicts that the number of species which form part of a particular community and have similar fundamental niches is evolutionary and ecologically constrained (Hutchison 1957; May & MacArthur 1972; Holt 2009). The limitation is due to the fact that these species exploit the same or at least similar kind of resources and low levels of resources in high-diversity communities make them resistant to invasions (Tilman 2004; Ricklefs 2010). Interspecific competition is thought to place the limit on the number of species which can coexist and resource partitioning in habitat dimensions seems to be more important than in food-type dimensions and in temporal dimension (Schoener 1974, 1986). In fact, a recent analysis of the community structure of an African anuran assemblage supports the structuring role of specific habitat affinities, but fails to detect significant resource partitioning due to interspecific competition (Behangana & Luiselli 2008). Tropical amphibian communities are of particular interest to test predictions of stochastic niche theory (Tilman 2004) because they are species-rich and the strict dependence of anuran reproduction on rainfall may lead to high temporal and spatial competition (Crump 1974). Amphibians provide examples of spatial, diel and seasonal resource partitioning with respect to advertisement call properties, oviposition sites, and diel call activity, enabling species with similar fundamental niches to coexist (e.g. Crump 1974; Toft 1985; Garcia-Rutledge & Narins 2001, Gottsberger & Gruber 2004; Prado *et al.* 2005; Boquimpani-Freitas *et al.* 2007). In reproductive anurans, the acoustic niche integrates a spatial dimension, i.e. the microhabitat

used for calling, a temporal dimension, i.e. the time of day when calling takes place, and a call structure dimension, i.e. the physical features of the advertisement call. Therefore, we predict that in species-rich anuran communities realized acoustic niches are narrow with little overlap to minimize acoustic interference, thus facilitating mating of conspecifics by acoustic resource partitioning. Moreover, species saturation of the community should lead to a more or less even distribution of niche breadth (Tilman 2004). While temperate-zone anuran communities comprise considerably less than five syntopic species on average, tropical assemblages are usually much richer in frog and toad taxa (Wells 2007). In lowland Amazonia (South America), 53 species were found on average (maximum: 87, Santa Cecilia, five localities reviewed by Duellman 1978 and Toft & Duellman 1979), and in the lowland and montane regions of the Albertine Rift (Africa) average species richness amounts to 20 taxa (maximum: 65, Virunga National Park, 27 localities reviewed by Plumptre *et al.* 2007). To test predictions about acoustic niche breadth and overlap, we chose an Albertine Rift community inhabiting a cultivated wetland at 1645 m a.s.l., a typical habitat at the foot of hills throughout Rwanda and Burundi. Preliminary surveys on anuran species richness suggest the presence of at least 10 taxa, indicating the suitability of the study site for comparative niche analysis in an Afromontane community (Sinsch *et al.* 2011). Since regional hydrographical systems ensure connectivity of cultivated wetlands over hundreds of kilometres, local anuran communities may be saturated due to frequent invasions by dispersing individuals (Tilman 2004). Therefore, our study aimed (1) to assess the identity of the local taxa using morphological, bioacoustic and molecular features, (2) to estimate the actual species richness of the community using rarefaction methods, (3) to quantify breadth and overlap of the specific acoustic niches and the partial contribution of spatial, diel and call structure dimensions, and (4) to evaluate potential acoustic niche partitioning by comparing actual with random niche overlap.

Materials and Methods

Study area

Species diversity and diel vocalization activity of a montane anuran community were studied in a cultivated wetland (‘marais’) near Butare, Rwanda (2.60059°S, 29.75964°E, 1645 m

a.s.l.). The study area had a north–south extension of 4.45 km and an average width of 150–200 m. Within the study area, we identified seven microhabitat types which were used by amphibians: partially flooded rice fields (34% of total area), uncultivated swamp (9%), dry pasture/crops (4%), sedgy area (24%), central ditch (>1 m width; 2%), surface irrigation channels (20–30 cm width; 1%), and fish ponds (6%). The remaining area included buildings, streets and other unsuitable habitats for amphibians. Bioacoustic surveys used for niche analysis were performed in four areas of about 40 000 m² which covered all microhabitat types in varying proportions and were located almost equidistant along the north–south axis of the study area.

Rarefaction analysis of species richness

Species identification: Basic to niche analysis was a thorough assessment of the local number of amphibian exploiting the marsh habitat. Species identification was based on external morphological features (mainly colour pattern, shape and size), on tissue samples used for DNA barcoding, and on advertisement call structure. Nomenclature follows Frost (2011).

Tissue samples preserved in 98% ethanol were used to sequence a fragment of the 16S mitochondrial rRNA gene, a suggested universal marker to barcode amphibians for species allocation (Vences *et al.* 2005).DNA was extracted 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 16S mitochondrial rRNA using standard primers (Palumbi *et al.* 2002) and standard PCR conditions (Palumbi 1996) with the following thermal cycle profile: 120 s at 94°C, followed by 33 cycles of 94°C for 30 s, 53°C 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 Highpure PCR Product Purification Kit (Roche Diagnostics). Sequencing was performed with the DYEnamic ET Terminator Cycle Sequencing Premix kit (GE Healthcare, Munich, Germany) for sequencing reactions run on a MegaBACE 1000 automated sequencer (GE Healthcare). DNA sequences were corrected and aligned by eye. Editing and analyses of pairwise distances were completed inMEGA5 (Tamura *et al.* 2011). The obtained sequences were compared with those in GenBank using a standard nucleotide–nucleotide BLAST search (Benson *et al.* 2004). Advertisement calls were recorded with a Sony™ PCM–D50 Linear PCM Recorder with stereo microphones, Sony Deutschland

GmbH, Cologne. We prepared sonograms and oscillograms of vocal records using Adobe Audition 3.0. Stereo recordings were converted to mono at a sampling rate of 44.1 kHz and resolution of 16 bits. Sonograms and frequency analyses were obtained applying Blackman-Harris Fast Fourier transformation with a FFT size of 1024 Hz. Call structure was characterized by measuring call duration (ms), pulses per call, pulse rate (Hz), pulse duration (ms), interpulse interval (ms) and dominant frequency (Hz). Based on previous records of identified specimens and call descriptions in literature (Schiøtz 1999; Channing & Howell 2006; du Preez & Carruthers 2009) we assigned calls post hoc to species. Advertisement calls are known to be species-specific in anurans (reviewed by Schneider & Sinsch 2007).

Sampling design: Species composition of the local frog community was assessed during 15 visual and bioacoustic surveys (3–8 man hours each, starting at 17:00 and ending at 23:00). Survey dates were 3, 4, 5, and 6 of March 2009 (beginning of the first annual rainy season), 4, 5, 7, 8, 13, 16 and 17 October 2009, and 6, 8, and 10 September 2010 (beginning of the second annual rainy season). Visual surveys were usually non-invasive and species identification was based on external morphological features, whenever possible. If visual identification remained inconclusive (e.g. *Ptychadena* individuals), the specimen was collected for morphometric and molecular species identification. Vocalizations of all specimens advertising in a given microhabitat were recorded for at least 10 minutes.

Statistical analysis: Descriptive statistics depended on the outcome of the Shapiro-Wilks test for normality. Normally-distributed data were described by the arithmetic mean and corresponding standard error, and those deviating significantly from normality by median and range. Significance level was set at alpha = 0.05. All calculations were based on procedures in the program package Statgraphics Centurion for Windows, version XV. Species detected during each survey (results of combined visual and acoustic assessment) were coded as incidence data (presence = 1, absence = 0), independent of the actual number of specimens seen or of calls recorded.We chose presence/absence analysis because there is no standardized method to obtain a reliable estimate of abundance using our survey methods. Non-invasive visual surveys underestimate the actual abundance of species with little surface activity and cryptic colouration (e.g. *Kassina* and *Phrynobatrachus*), bioacoustic estimates confound specific call repetition rates (e.g. high in *Hyperolius* sp. 3 (‘viridiflavus’), low in *H. kivuensis*) with abundance. Presence/absence data matrix (15 species vs 15

surveys) was submitted a Cole-rarefaction analysis and a jackknife richness estimation using the statistical package EstimateS 8.20 (Colwell 2009).

Acoustic niche breadth and overlap

We consider three niche dimensions which may contribute to acoustic resource partitioning in anuran communities: (1) location utilized for advertisement, (2) diel timing of calling activity, and (3) spectral and temporal structure of advertisement call. The spatial niche dimension includes seven resource classes, i.e. the microhabitats in which calling males were detected. The temporal niche dimension comprises six resource classes representing the hours of daylight (17:00), twilight (18:00) and darkness (19:00–22:00). The advertisement call structure provided 45 resource classes, of which 23 are dominant frequency intervals (width: 200 Hz, range: 600–5000 Hz), and 22 are pulse rate intervals (width: 10 Hz; range: 10–220 Hz). The rationale of choosing these features as representatives of call structure is that exploiting different frequency bands improves distinction of conspecific calls from background noise including other frog species and that pulse rate is crucial for the recognition of conspecifics in many species (e.g. Martino & Sinsch 2002).

Sampling design: During the October study period of 2009 the four 40 000 m² areas were sampled two days each. Every hour between 17:00 and 22:00 we recorded calling activity in each microhabitat type for two minutes. Air temperature (to the nearest 0.1°C) was registered in each microhabitat (usually at 1.0 m above-ground) immediately after recording using a TinyTag temperature logger (Gemini Data Loggers Ltd, Chichester, U.K.) Ambient humidity was roughly estimated using the semi-quantitative scale 0=rainfall at the day of recording, –1, –2, and –3=rainfall one, two, or three days before recording.

Statistical analysis: Call activity was defined as number of calls given by the males of a given species in a predetermined microhabitat within one minute irrespective of the actual number of males producing these calls. As call activity was influenced by time of the day and microhabitat type (fixed factors) and by temperature and humidity (covariates), we run an ANCOVA on the raw data of each species and used the least square means obtained as the adjusted call activity for the spatial and temporal resource classes. Dominant frequency and pulse rate distributions were calculated based on the analysis of 44–167 calls per species.

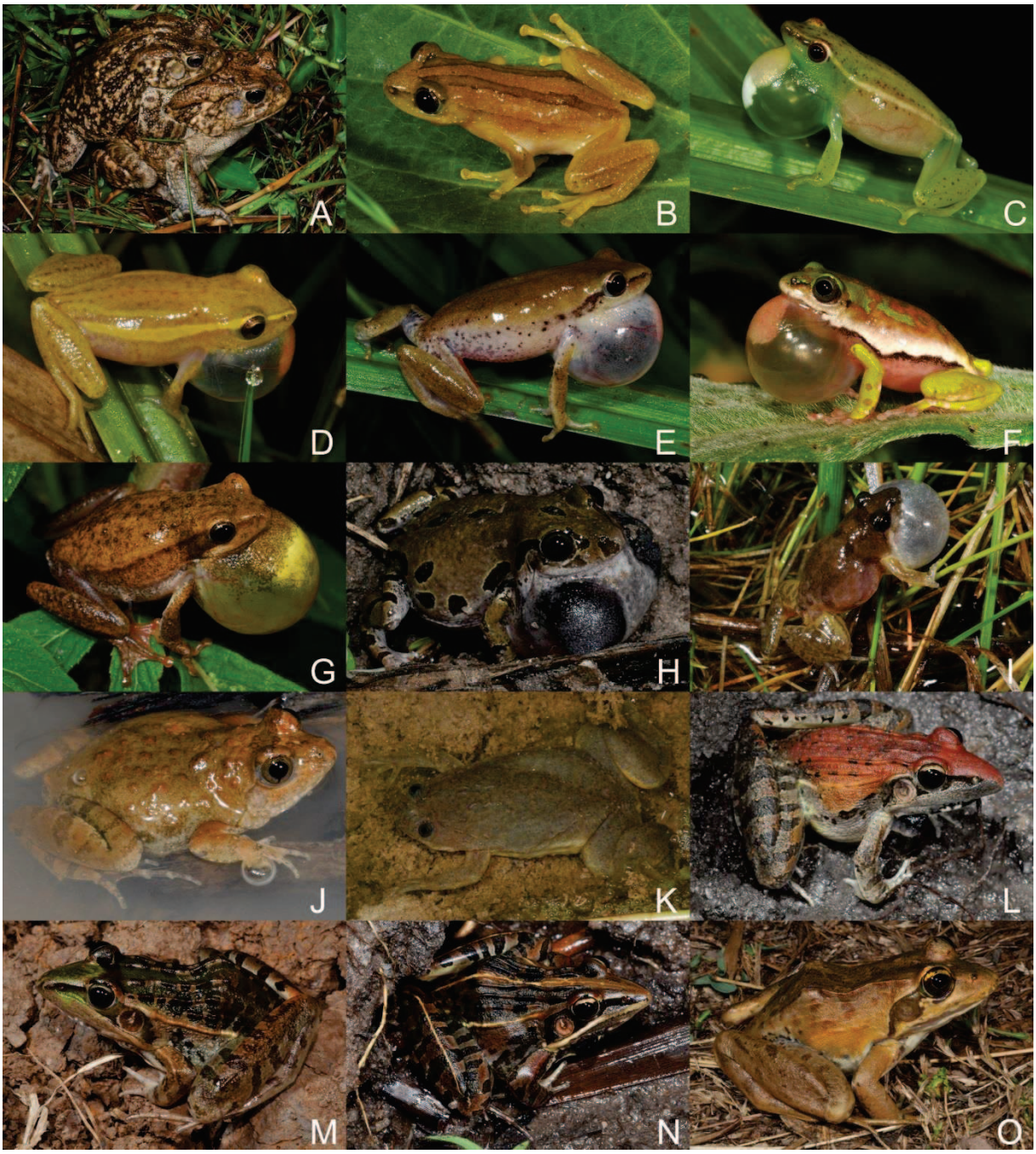

Figure 2. Anuran species detected during visual surveys in the swamps of Butare. **A)** *Amietophrynus regularis*, **B)** *Afrixalus quadrivittatus*, **C)** *Hyperolius* sp. 1 (*nasutus*-group), **D)** *Hyperolius* sp. 2 (*cinnamomeoventris*-group), **E)** *Hyperolius kivuensis*, **F)** *Hyperolius lateralis*, **G)** *Hyperolius* sp. 3 (*viridiflavus*-group), **H)** *Kassina senegalensis*, **I)** *Phrynobatrachus* sp. 1 (*mababiensis*-group), **J)** *Phrynobatrachus* sp. 2 (*natalensis*-group), **K)** *Xenopus victorianus*, **L)** *Ptychadena anchietae*, **M)** *Ptychadena* cf. *mascareniensis*, **N)** *Ptychadena porosissima*, **O)** *Amietia* sp. 1 (*angolensis*-group).

Niche breadth (NB) was estimated as the inverse of Simpson's diversity index (Pianka 1986) and standardized to a range between 0 and 1 as NB* = (NB – 1)/(r – 1), with r = the number of resource classes. Specialists with NB* = 0 exploit only one resource class, generalists with NB* = 1 use all resource classes. NB* was calculated for each niche dimension separately,

and also for the complete acoustic niche. Average niche breadth was calculated as the arithmetic mean and corresponding standard deviation. Pairwise acoustic niche overlap was estimated following Colwell and Futuyama (1971). To test for resource partitioning, we compared the average measured niche overlap $NO_{empirical}$ (n=91) with the theoretical niche overlap NO_{random} which would occur in the absence of resource partitioning. NO_{random} was calculated as the average of 10 random number generations within the range 0 to 1. Data scatter was compared using the corresponding standard deviations of average niche overlap. A cluster analysis was run on the matrix of niche overlap using the complete linkage procedure to evaluate the magnitude of acoustic resource partitioning among the species.

Results

Community composition

A total of 15 anuran species were detected during visual surveys (Figure 2), whereas a subset of 14 was acoustically detected as well (Figure 3, Table 1). The aquatic *Xenopus victorianus* calls exclusively below the water surface and remains undetected by conventional recording devices for air-borne sounds (Tobias *et al.* 2004; M.L. Tobias, pers. comm.). Molecular evidence based on mitochondrial 16S rRNA gene sequences corroborated the presence of 15 well-differentiated taxa which coincided with the morphologically and bioacoustically identified species. GenBank search and advertisement call structure were used for taxonomic identification of the species. Eight taxa collected agreed in all studied features with the nominal species *Xenopus victorianus*, *Amietophrynus regularis*, *Ptychadena anchietae*, *P. porosissima*, *Kassina senegalensis*, *Afrixalus quadrivittatus*, *Hyperolius kivuensis* and *H. lateralis* (Frost 2011). Three further species of *Hyperolius* differed considerably from all gene sequences available but were assignable to known species groups (*nasutus*,*viridiflavus*). The five species of *Hyperolius* differed from each other and from *Afrixalus quadrivittatus* with respect to uncorrected p distances at 9.4–18.3% among each other (Table 2). Besides two identified *Ptychadena* species, external morphology, bioacoustics and uncorrected p distances agreed that a third taxon (*P*. cf. *mascareniensis*, Bwong *et al.* 2009) was present: *P. anchietae* vs. *P.* cf. *mascareniensis* 13.8% p distance, *P. anchietae* vs *P. porosissima* 10.5%, and *P.* cf. *mascareniensis* vs *P. porosissima* 13.3%.

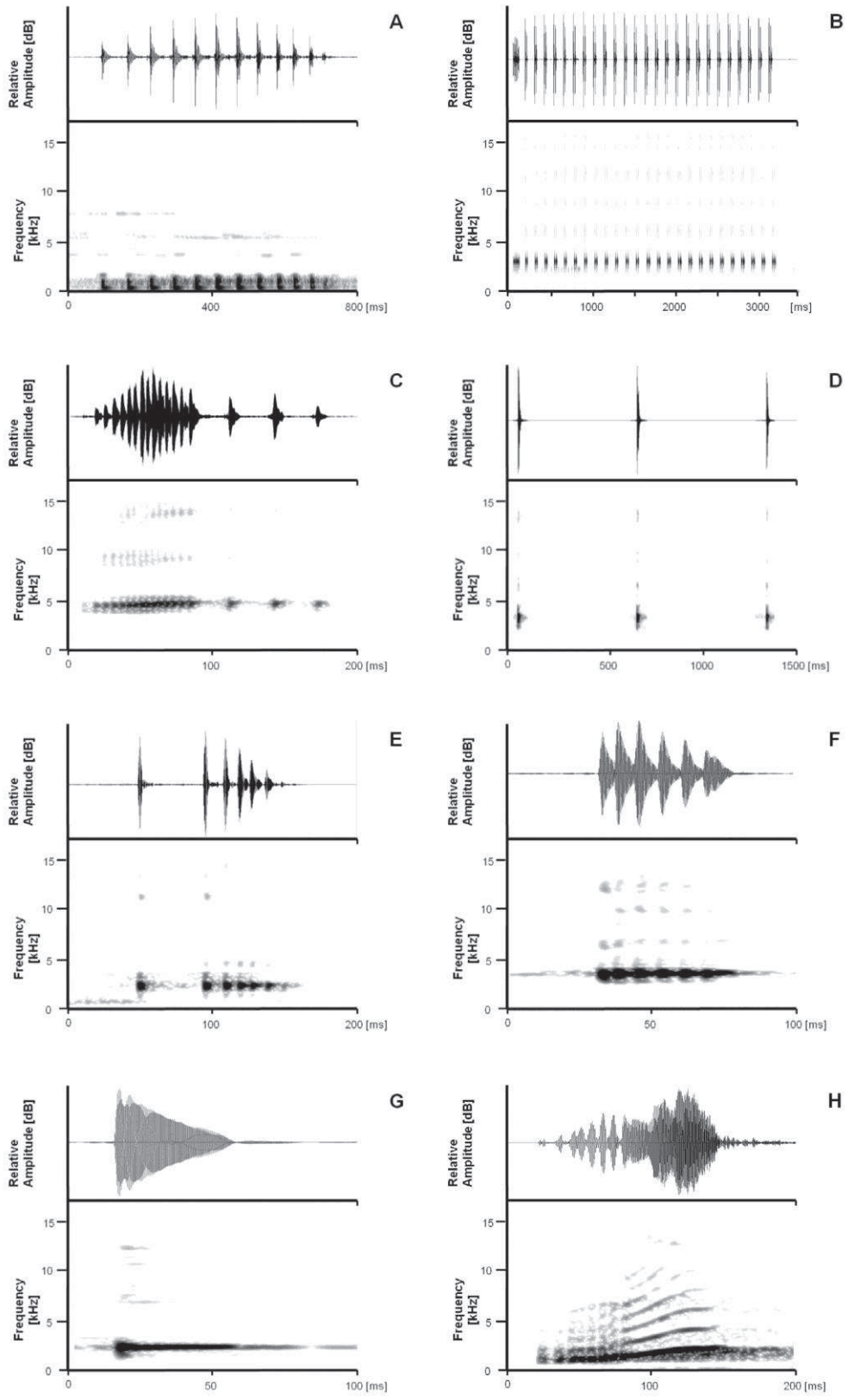

Figure 3. Oscillogram and sonogram of advertisement calls corresponding to 14 species of the anuran community inhabiting the swamps of Butare. **A**, *Amietophrynus regularis*, air temperature when recorded 20.5°C, **B**, *Afrixalus quadrivitattus*, 19.0°C, **C**, *Hyperolius* sp. 1 (*nasutus*-group), 17.5°C, **D**, *Hyperolius* sp. 2 (*cinnamomeoventris*-group), 17.5°C, **E**, *Hyperolius kivuensis*, 18.5°C, **F**, *Hyperolius lateralis*, 17.5°C, **G**, *Hyperolius* sp. 3 (*viridiflavus*-group), 17.5°C, **H**, *Kassina senegalensis*, 21.5°C.

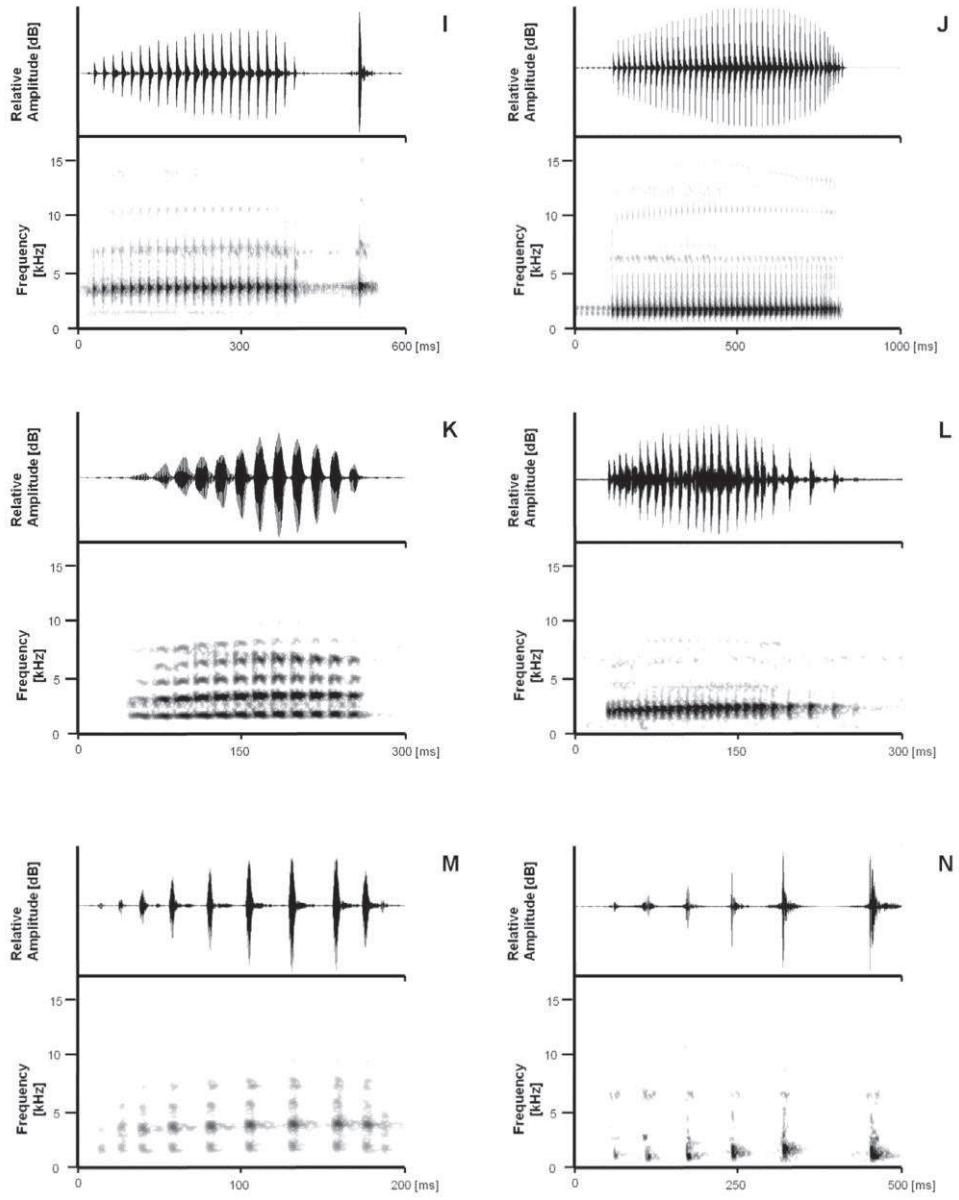

Figure 3. (continued) I) *Phrynobatrachus* sp. 1 (*mababiensis*-group), 18.5°C, **J)** *Phrynobatrachus* sp. 2 (*natalensis*-group), 19.0°C, **K)** *Ptychadena anchietae*, 19.5°C, **L)** *Ptychadena* cf. *mascareniensis*, 19.0°C, **M)** *Ptychadena porosissima*, **N)** *Amietia* sp. 1 (*angolensis*-group), 19.0°C.

Moreover, we found two species of *Phrynobatrachus* (14.6% uncorrected p distance) which differed from the described species, but were assigned to the *natalensis*-species group and to the *mababiensis*-group (Zimkus *et al.* 2010), respectively. The anuran community also included an *Amietia* species, which was assigned to the *angolensis*-species group, but differed considerably from the nominal species with respect to call structure and p distance. Taxonomic assignment of these taxa will be reported elsewhere.

Table 1. Features of advertisement calls produced by the 14 anuran species with air-borne calls. *Xenopus victorianus*, which vocalizes below the water surface, is not considered. Data are given as medians and the corresponding ranges (minimum–maximum) recorded within a temperature interval of 16–21°C.

Species	Call duration (ms)	Pulses per call (*n*)	Pulse rate (Hz)	Pulse duration (ms)	Interpulse interval (ms)	Dominant frequency (Hz)
Amietophrynus regularis	719 (525–1011)	12 (8–17)	17.2 (13.3–19.9)	24 (13–37)	40 (24–58)	388 (301–510)
Afrixalus quadrivittatus	2855 (859–7 598)	68 (17–138)	23.9 (15.5–9.9)	6 (3–8)	7 (4–11)	3251 (3078–3511)
Hyperolius kivuensis	75 (50–107)	5 (4–7)	64.1 (46.7–95.9)	3 (1–5)	7 (3–23)	2649 (2177–3124)
Hyperolius lateralis	27 (13–42)	6 (4–8)	250.0 (193.5–368.4)	3 (1–4)	1 (0–3)	3500 (3046–3854)
Hyperolius sp. 1 (*nasutus*-group)	139 (62–287)	18 (13–23)	129.5 (70.6–209.7)	3 (2–4)	2 (0–106)	4621 (4005–5272)
Hyperolius sp. 2 (*cinnamomeoventris*-group)	664 (550–2 003)	6 (5–12)	9.0 (4.5–10.8)	3 (1–5)	0 (0–593)	3246 (3083–3992)
Hyperolius sp. 3 (*viridiflavus*-group)	47 (24–82)	1	–	47 (24–82)	–	2640 (2220–2917)
Kassina senegalensis	118 (58–159)	1	–	118 (58–159)	–	1076 (810–1421)
Phrynobatrachus sp. 1 (*mababiensis*-group)	374 (421–584)	22 (15–28)	61.5 (33.6–69.3)	7 (3–10)	9 (5–12)	3809 (3488–4306)
Phrynobatrachus sp. 2 (*natalensis*-group)	632 (501–821)	42 (34–58)	67.1 (56.4–79.6)	8 (6–10)	7 (4–9)	1490 (1292–1840)
Ptychadena anchietae	220 (110–278)	11 (7–14)	52.6 (36.4–64.3)	10 (8–13)	5 (3–8)	3359 (3014–3832)
Ptychadena cf. *mascareniensis*	189 (72–281)	17 (8–26)	94.0 (74.1–111.1)	5 (3–7)	4 (2–6)	2239 (1772–2756)
Ptychadena porosissima	182 (100–262)	10 (5–13)	52.9 (41.3–84.4)	6 (3–12)	13 (7–20)	4091 (3660–4565)
Amietia sp. 1 (*angolensis*-group)	282 (65–633)	5 (2–10)	17.9 (13.1–30.8)	6 (3–14)	65 (52–87)	1421 (818–1765)

Table 2. Mean uncorrected p distances [%] of the 16S mitochondrial rRNA gene (550 bp sequences) of sympatric *Hyperolius* and *Afrixalus* species inhabiting an agricultural wetland at Butare.

Species	*H. kivuensis*	*H. lateralis*	*H.* sp. 1	*H.* sp. 2	*H.* sp. 3
Afrixalus quadrivittatus	17.7	18.3	14.3	17.9	17.0
Hyperolius kivuensis	–	9.4	16.4	11.4	13.5
Hyperolius lateralis		–	15.5	10.0	13.4
Hyperolius sp. 1 (*nasutus*-group)			–	15.7	16.3
Hyperolius sp. 2 (*cinnamomeoventris*-group)				–	13.6
Hyperolius sp. 3 (*viridiflavus*-group)					–

Daytime visual surveys were more effective than simultaneous acoustic surveys because only three species (*Kassina senegalensis*, *Phrynobatrachus* sp. 1 and 2) occasionally produced advertisement calls (Table 3). However, visual species detection during day was limited to mainly juveniles, few adults and many tadpoles of *Ptychadena* spp. and *Amietia*, occasionally *Phrynobatrachus* and *Xenopus* adults and almost never hyperoliids. Following sunset, detection probability reversed because, depending on the microhabitat sampled, 5–12 species vocalized simultaneously. Visual detection usually followed acoustic detection and subsequent homing-in to the origin of sound. Consequently, rapid assessment of local species richness was most effective between 19:00 and 22:00 h, using a combination of visual and acoustic survey. Rarefaction analysis with subsequent jackknife estimation of expected species richness confirmed that the 15 species collected during 15 systematic surveys are most probably all species present in the marais of Butare (Figure 4). Single surveys yielded 7 to 12 species, i.e. on average 66.7% of the total species richness. Species which were rarely observed were *Hyperolius* sp. 1 (2/15 surveys), *H. lateralis* (4), and *H.* sp. 2 (5). Following 4–5 surveys, at least 93% of species had been collected and jackknife estimates indicated the correct total species number.

Acoustic niche breadth and overlap

Standardized niche breadth NB*call structure showed high specialization among all species, ranging from 0.04 to 0.16 with an average of 0.09±0.05 (Table 4). Assuming an equally partitioned niche space among the 14 species considered, average niche breadth would amount to 0.07. By contrast, $NB^*_{spatial}$ values were far more heterogeneous, ranging from 0 in *H.* sp. 1 and *H.* sp. 2, which exclusively advertised in the uncultivated swamp areas, to 0.41–0.44 in *H. kivuensis*, *A. regularis* and *Phrynobatrachus* spp. calling in 4–7 microhabitat

Table 3. Spatial and temporal features of the acoustic niches in an anuran community of the Butare wetland. n.a. = no information available.

Species	Preferred habitat	Additional habitats	Calling position	Peak calling activity	Suppl. calling activity
Amietophrynus regularis	Rice field	Central ditch, uncultivated swamp	On ground	7–10 pm	–
Afrixalus quadrivittatus	Rice field	Irrigation channel	Above ground	6–7 pm	–
Hyperolius kivuensis	Uncultivated swamp	Rice field, irrigation channel	Above ground	9–10 pm	7–8 pm
Hyperolius lateralis	Uncultivated swamp	Rice field, irrigation channel	Above ground	6–9 pm	–
Hyperolius sp. 1 (*nasutus*-group)	Uncultivated swamp	–	Above ground	7–9 pm	–
Hyperolius sp. 2 (*cinnamomeoventris*-group)	Uncultivated swamp	–	Above ground	8 pm	–
Hyperolius sp. 3 (*viridiflavus*-group)	Uncultivated swamp	Central ditch, Pond	Above ground, on water surface	7–10 pm	–
Kassina senegalensis	Rice field	Irrigation channel, uncultivated swamp	On ground	6–7 pm	5 pm, 8–10 pm
Phrynobatrachus sp. 1 (*macabiensis*-group)	Rice field	Pasture, irrigation channel	On ground	8–10 pm	5–7 pm
Phrynobatrachus sp. 2 (*natalensis*-group)	Rice field	Irrigation channel	On ground	6–10 pm	5 pm
Ptychadena anchietae	Rice field	Irrigation channel	On water surface	7–8 pm	6 pm, 9–10 pm
Ptychadena cf. mascareniensis	Rice field	Irrigation channel	On water surface	7–8 pm	6 pm, 9–10 pm
Ptychadena porosissima	Rice field	Irrigation channel	On water surface	7–8 pm	6 pm, 9–10 pm
Amietia sp. 1 (*angolensis*-group)	Sedges	Central ditch	On ground, on water surface	8–10 pm	–
Xenopus victorianus	Pond	Irrigation channel	Below water surface	n.a.	n.a.

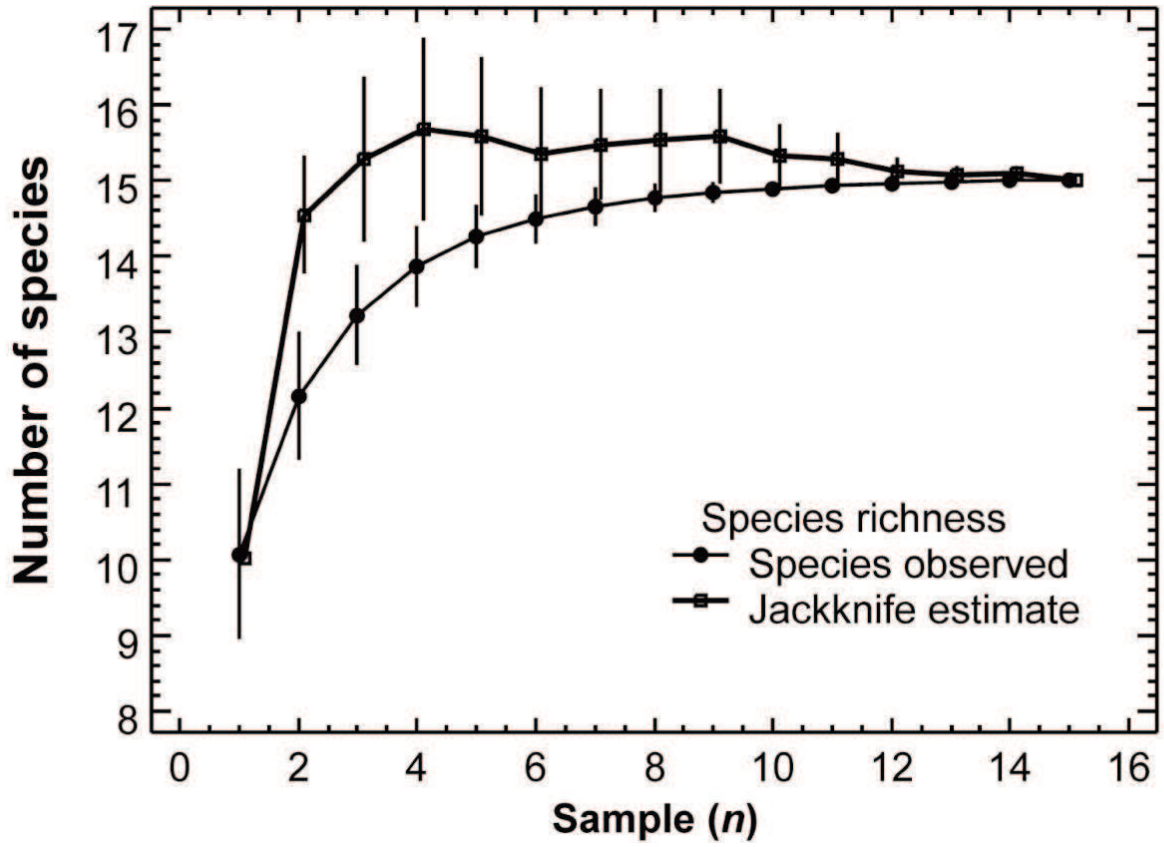

Figure 4. Rarefaction analysis of anuran species richness based on 15 combined visual and bioacoustic surveys. Symbols represent frequency estimates, and vertical bars corresponding standard deviations.

types. Nevertheless, the distribution of $NB^*_{spatial}$ did not deviate significantly from a normal distribution, yielding an average niche breadth of 0.18 ± 0.17. The largest heterogeneity was observed in $NB^*_{temporal}$ with 0 in *H.* sp. 2 and 0.93 in *P.* sp. 1. The latter species called at any hour during the survey period, while the first was heard only at 20:00. Again, distribution of $NB^*_{temporal}$ did not deviate significantly from a normal distribution, yielding an average niche breadth of 0.49 ± 0.21. Combining all components of the acoustic niche to calculate NB^*_{Total} resulted in a narrow niche breadth (0.04–0.13), mainly due to the contribution of the species-specific advertisement call structure. Average NB^*_{Total} was 0.08 ± 0.04. The pairwise standardized niche overlap did not differ significantly from a normal distribution (Shapiro-Wilks test, W= 0.96, P = 0.056). Average measured niche overlap was $NO_{empirical}$ = 0.30 ± 0.12. Maximum niche overlap amounted to 0.678 in the species pair *P. anchietae* and *P. porosissima*, which differed exclusively in advertisement call structure, while minimum overlap was 0.065 between *Hyperolius* sp. 2 and *Phrynobatrachus* sp. 1 (Figure 5). Assuming a uniform and equidistant distribution of the 14 acoustic niches (i.e. maximum partitioning),

Table 4. Standardized niche breadth NB* of the single dimensions (temporal, spatial and call structure) of the acoustic niche. NB^*_{total} is the breadth of the complete niche using an additive calculation model.

Species	$NB^*_{Temporal}$	$NB^*_{Spatial}$	$NB^*_{Call\ structure}$	NB^*_{Total}
Amietophrynus regularis	0.51	0.43	0.04	0.12
Afrixalus quadrivittatus	0.20	0.27	0.05	0.11
Hyperolius kivuensis	0.52	0.41	0.10	0.11
Hyperolius lateralis	0.57	0.05	0.06	0.04
Hyperolius sp. 1 (*nasutus*-group)	0.57	0	0.21	0.04
Hyperolius sp. 2 (*cinnamomeoventris*-group)	0	0	0.07	0.05
Hyperolius sp. 3 (*viridiflavus*-group)	0.68	0.27	0.08	0.10
Kassina senegalensis	0.41	0.26	0.06	0.08
Phrynobatrachus sp. 1 (*macabiensis*-group)	0.93	0.44	0.12	0.13
Phrynobatrachus sp. 2 (*natalensis*-group)	0.58	0.10	0.07	0.07
Ptychadena anchietae	0.44	0.02	0.09	0.04
Ptychadena mascareniensis	0.43	0.03	0.16	0.06
Ptychadena porosissima	0.49	0.04	0.12	0.05
Amietia sp. 1 (*angolensis*-group)	0.56	0.15	0.07	0.13

the expected average overlap $NO_{uniform}$ would amount to 0.11. By contrast, calculation of random niche overlap NO_{random} (i.e. absence of resource partitioning) yielded values varying between 0.47 ± 0.20 and 0.55 ± 0.18 with a second-order mean of 0.51 ± 0.19. Average measured niche overlap $NO_{empirical}$ was significantly lower than random niche overlap NO_{random} (second-order mean; t-test, t=–9.32, $P < 0.0001$) and standard deviation of the empirical distribution as a measure of scatter was also lower (F-test, $F = 0.42$, $P < 0.0001$).

Discussion

Species richness of Afromontane anuran communities

Amphibian diversity decreases from lowland to Afromontane communities, as evidenced in East Africa (Poynton *et al.* 2007; Plumptre *et al.* 2007; Sinsch *et al.* 2011) and in southeastern Africa (Poynton & Boycott 1996). Altitudinal changes in species richness are usually associated with marked changes in species composition distinguishing between lowland (roughly below 1 000m a.s.l.), mid-elevation (1000–2000 m a.s.l.) and high-altitude communities (usually above 2000 m a.s.l.; e.g. Loveridge 1937; Poynton & Boycott 1996;

Menegon & Salvidio 2005; Poynton *et al.* 2007; Sinsch *et al.* 2011). Owing to the low number of surveys per sampled area and the unsettled taxonomy of many anuran species groups (e.g. *Ptychadena* spp., Bwong *et al.* 2009; *Phrynobatrachus* spp., Zimkus *et al.* 2010), local species numbers and identity may include a wide margin of error (e.g. Menegon & Salvidio 2005; Loader *et al.* 2011). Even so, mid-elevation communities at different latitudes typically comprise between 11 and 24 species (Poynton & Boycott 1996; Menegon & Salvidio 2005), indicating that species saturation is reached at this diversity level. Rarefaction and jackknife analyses confirmed that 15 anuran species constitute the complete current species richness in the Butare community, and preliminary surveys in comparable cultivated and pristine wetlands on the Rwandan plateau suggest that species richness does not vary significantly among localities (J.M. Dehling, unpubl. obs.). However, almost half of the species collected

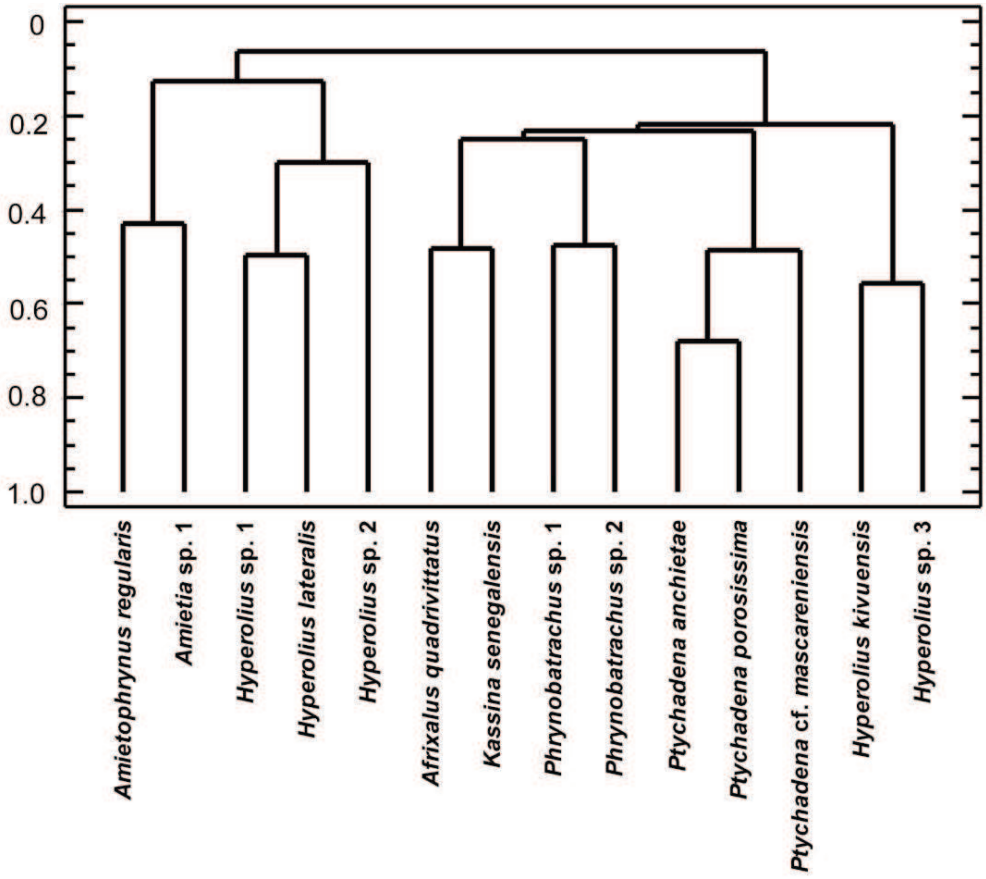

Figure 5. Cluster analysis (method: complete linkage) of bioacoustic niche overlap of 14 species comprising the anuran community of the swamps near Butare. *Xenopus victorianus* was not included due to missing data, but its niche differs from all others due its unique subaquatic mode of advertising. Breadth of the specific bioacoustic niche and of its temporal, spatial und call feature components is summarized in Table 4.

are currently undescribed or considered synonyms of other species, suggesting a significant

cryptic diversity in Afromontane frog communities. The surprising high rate of previously unrecognized diversity is due to the formerly common assignment of morphologically similar anurans to a single morphospecies which often actually represents a complex of different species (e.g. *Hyperolius cinnamomeoventris*, *nasutus*, *viridiflavus* species groups; Schiøtz 1999), as evidenced by molecular and bioacoustic characters.

Independent of the taxonomic assignment to new or already described species, all taxa in the marais communities are easily distinguishable based on morphological, bioacoustic and molecular features. In this habitat type, with occasional shrubs as the tallest vegetation, sampling effort needed to reliably quantify anuran diversity amounts to only five thorough surveys. Given the connectivity of marais habitats throughout Rwanda and probably Burundi, we conclude that the Butare community is probably species-saturated. Thus, this anuran community fulfils the requirements (high species richness, species saturation) to test the predictions made by stochastic niche theory for resource partitioning with respect to the acoustic niche.

Actual and predicted resource partitioning

Niche assembly in the Butare frog community agrees with predictions of theory in that niche breadth (NB^*_{Total}) was actually as narrow as predicted for species-saturated communities (0.08 vs 0.07) and that observed niche overlap was significantly lower than expected. Variation of niche breadth among the 14 terrestrially advertising anurans was correspondingly small, indicating selection for narrow acoustic niches. Minor spatial and temporal segregation of advertisement activity do not suggest strong specialization among species, probably due to common ecological and physiological constraints such as water-bodies for successful development of their offspring and high relative air humidity to minimize evaporative water loss. Consequently, the less constrained acoustic dimension is the advertisement call structure, which is selected for uniqueness among syntopic species and determines the reproductive success among conspecifics (e.g. Lode & Le Jacques 2003; Schneider & Sinsch 2007).

Just as niche breadth and low interspecific overlap, acoustic resource partitioning indicates a long period of coevolution which we did not expect in a community inhabiting a cultivated wetland subject to strong human impact. However, undisturbed marais habitats show a similar community structure (J.M. Dehling, unpubl. data) and we assume that progressive

cultivation and probably expansion of area during previous centuries have promoted connectivity and homogenization of marais communities. Cultivated wetlands probably provide more suitable habitats for anurans than pristine ones because they are highly fragmented by small-sized fields that are separated by an extensive system of irrigation channels. If the magnitude of overlap is an estimator of coexistence period, duration of sympatry of the species constituting the marais community probably differs. *Ptychadena* species with widely overlapping acoustic niches may represent relatively recent invasions of two of the three species, whereas *Hyperolius* sp. 1 (cinnamomeoventris group) with an average overlap of 0.19 in all species pairs but the one with *H. lateralis* (0.46) shows a niche segregation closely approaching the minimum theoretical overlap. Future studies of the community structure in isolated wetlands should reveal whether or not the number of local *Ptychadena* species is fewer than three, as would be expected under the assumption of human-mediated dispersal.

A further constraint of acoustic resource partitioning is probably the limited frequency range available for an anuran advertisement call (e.g. Garcia-Rutledge & Narins 2001; Preininger *et al.* 2007; this study). Few anurans are capable of emitting calls at a dominant frequency above 5 kHz because of the morphology of the sound-producing apparatus (Wells 2007; Böckle *et al.* 2009). Moreover, at the study site the frequency range below 5 kHz is not only used for anuran advertisement, but also by at least ten nocturnal, unidentified insect species (crickets, bugs; U. Sinsch, unpubl. data). Avoidance of masking background noise probably leads to a compression of available acoustic niche space, and thus to an increased overlap of occupied niches in species-rich communities.

In conclusion, stochastic niche theory predicts rather accurately the acoustic resource partitioning observed in the anuran community of the Butare wetland. Available acoustic niche space appears to be fully occupied by the 15 species currently present, and the wide overlap of *Ptychadena* spp. niches raises doubt about whether local coexistence will be possible in the long term without further niche segregation. Ongoing research on acoustic niche partitioning in mid-elevation wetlands with lower species richness and a distinct species composition is aimed at analysing the variability of realized acoustic niche breadth in response to distinct competition scenarios.

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III

Diversity of Ridged Frogs (Anura: Ptychadenidae: *Ptychadena* spp.) in wetlands of the upper Nile in Rwanda: Morphological, bioacoustic, and molecular evidence

This chapter has been published as:

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Abstract

We investigated the distribution of species of the genus *Ptychadena* at nine sites at the upper Nile and its catchment in Rwanda. For species delimitation, we chose an integrative approach, combining morphological and bioacoustic data and DNA barcoding (mitochondrial 16S rRNA gene). We identified three species using independent evidence from the three different data sets: *Ptychadena anchietae*, *Ptychadena porosissima*, and a species of the *Ptychadena mascareniensis* group. The latter is undistinguishable genetically, bioacoustically, and morphologically from populations from Uganda, Kenya, and Egypt. We resurrect the name *Ptychadena nilotica* for these populations. The species differs strongly genetically from topotypic *P. mascareniensis*, and from clades referred to as *P.* cf. *mascareniensis* from Western and Central Africa. Morphologically, the three Rwandan species can be differentiated by their quantitative morphometrics (discriminant analysis, success rate: 98.3%) and by a number of qualitative characters of external morphology which are useful for identification in the field. The specific features of the advertisement call differ

unequivocally among the three species and allow detection and identification in the field. We also provide quantitative descriptions of temporal and frequency structure of the release calls of two of the species and the distress calls of all three species. Finally, we compare the 16S sequences obtained from Rwandan specimens with those deposited in GenBank to estimate geographical distribution of taxa in Africa.

Key words: Advertisement call, release call, distress call, *P. anchietae*, *P. mascareniensis*, *P. nilotica*, *P. porosissima*, DNA barcoding, integrative taxonomy

Introduction

Compared to other countries in East Africa like Tanzania, Kenya and Uganda (Channing and Howell, 2006), the herpetofauna of Rwanda is poorly known. Since 2009 we have been investigating the alpha diversity of the amphibian assemblages at numerous sites all over Rwanda and found several taxa which were unknown to science or with doubtful taxonomic assignment (Sinsch *et al.* 2011, 2012; Dehling 2012a; Channing *et al.* 2013). A notoriously challenging group are the Ridged Frogs of the genus *Ptychadena*. All species share a similar general appearance (sharp long snout, long legs, ridged dorsum) and colour polymorphism within each species can be substantial (e.g. Poynton 1970; Rödel 2000; Channing 2001; Channing and Howell, 2006). In the past, some species were described based on characters which later turned out to be too variable for taxonomic significance, several species names have been placed into the synonymy of a few nominal species, but were later recognized as distinctive species, and in few cases, even the type series of certain taxa are composed of specimens of more than one species (e.g. Boulenger 1879; Loveridge 1932; Laurent 1954; Guibé & Lamotte 1957; Schmidt & Inger 1959; Lamotte 1967; Poynton 1970). Thus, states of characters that potentially differ between species were mixed up in diagnoses, rendering them often useless. Approaches using standardized diagnostic schemes are rare (e.g. Perret 1979; Bwong *et al.* 2009) but have shown that species can be easily differentiable, especially when applying a combination of morphological, bioacoustic and/or genetic information (Passmore 1977; Perret 1979; Bwong *et al.* 2009). Often, two or more species occur in sympatry or even use the same microhabitats. Even when they can be distinguished locally, it is sometimes difficult to assign them to a certain species. Furthermore, phylogenetic

studies have shown that two or more genetically distinct lineages can be hiding under the same species name (Vences *et al.* 2004; Measey *et al.* 2007). Herein, we present the results of surveys in the wetlands along the upper Nile (Rukarara, Mwogo, Nyabarongo, Akagera) in southern and eastern Rwanda with regard to the occurrence of *Ptychadena* species. For species delimitation, we chose an integrative approach, combining morphological and bioacoustic data and DNA barcoding. Morphological diagnosis is based on quantitative morphometrics analyzed with multivariate statistics and on qualitative characters of external morphology useful in the field. The bioacoustic data set includes the quantitative descriptions of temporal and frequency structure of the advertisement calls, release calls, and distress calls. Finally, we compare the 16S rRNA sequences obtained from Rwandan specimens with those deposited in GenBank to estimate geographical distribution of taxa in Africa.

Materials and methods

Study areas

We investigated the distribution of species of the genus *Ptychadena* at nine sites at the upper Nile and its catchment in Rwanda: (1) Rukarara River (2°27.095’ S, 29°27.495’ E, 2031 m a.s.l.), (2) Mwogo River I (2°28.13’ S, 29°40.94’ E, 1576 m a.s.l.), (3) Mwogo River II (2°15.33’ S, 29°35.67’ E, 1537 m a.s.l.), (4) Nyabarongo River I (1°43.26’ S, 29°38.22’ E, 1410 m a.s.l.), (5) Nyabarongo River II/Kigali (1°57.82’ S, 30°00, 14’ E, 1358 m a.s.l.), (6) Nyabarongo River III/Mugesera (2°11.99’ S, 30°16.27’ E, 1330 m a.s.l.), (7) Akagera River (2°13.46’ S, 30°49.65’ E, 1293 m a.s.l.), (8) wetlands near Butare (2°36.0’ S, 29°45.4’ E, 1645 m a.s.l.; Sinsch *et al.*, 2012), and (9) wetlands near Gitarama (2°5.9’ S, 29°46.8’ E, 1810 m a.s.l.).

Identification of specimens

We identified three distinct advertisement call types in the field and traced the calls back to their sources. The advertising males were collected and examined morphologically. The initial set of specimens consisted of five males of each call type/morphospecies. Results of a morphological comparison were in congruence with the results of bioacoustics analyses. The presence of three distinct species was supported by a comparison of the partial sequence of

the mitochondrial 16S rRNA “barcoding” gene. For the bioacoustic and the morphological analyses we collected and recorded additional male specimens of each species which were identified by their advertisement calls and external morphology but were not genetically barcoded. Assignment of females to one of the three species is based on morphological similarity to the males and confirmed by comparison of the 16S gene sequence.

Morphological character definition and sampling

For a morphological analysis, a total of 50 *Ptychadena* adults (14 females, 36 males; Appendix 1) were collected in the field, euthanized, and preserved using 70% ethanol. They have been deposited in the collection of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK). We supplemented our data set with eight adult female specimens of *Ptychadena porosissima* (Steindachner 1867) from Rwanda (holotype and paratypes of its junior synonym *Ptychadena loveridgei* Laurent 1954) deposited in the collection of the Royal Museum of Central Africa, Tervuren, Belgium (Musée Royal de l’Afrique Central, MRAC). We took the following 18 measurements to the nearest 0.1mm using digital callipers: (1) Snout-vent length (SVL); (2) tibiofibula length (TFL, measured with both knee and tibio-tarsal articulation flexed); (3) thigh length (THL, from vent to knee with thigh being held vertically to median body plane and knee flexed); (4) total hindlimb length (LEG, from vent to tip of fourth toe with leg fully extended and being held vertically to median body plane); (5) tarsus + foot length (TarL, from tibio-tarsal articulation to tip of fourth toe); (6) foot length (FOT, from proximal end of inner metatarsal tubercle to tip of fourth toe); (7) forearm + hand length (ARM, distance from elbow to tip of third finger); (8) hand length (HND, distance from proximal end of inner palmar tubercle to tip of third finger); (9) head width (HW, measured at the level of the jaw joint); (10) head length (HL, distance from posterior end of mandible to tip of snout); (11) interorbital distance (IO, shortest distance between upper eyelids); (12) upper eyelid width (EW); (13) horizontal eye diameter (ED); (14) horizontal tympanum diameter (TD); (15) eye to nostril distance (EN, distance between anterior margin of eye and centre of nostril); (16) nostril to snout distance (NS, distance between centre of nostril and tip of snout); (17) snout length (SL, distance between anterior margin of eye to tip of snout); (18) internarial distance (NN, distance between centres of nostrils). To avoid an inter-observer bias, all measurements were taken by JMD. Morphometric data have been deposited at DRYAD (http://www.datadryad.org;

provisional http://dx.doi.org/10.5061/dryad.mm296). Additionally, we recorded the following qualitative characters: (1) position of external vocal sac aperture in males; (2) number of longitudinal dorsal dermal ridges; (3) texture of ventral skin; (4) extent of nuptial pads in males; (5) number of supernumerary metacarpal tubercles; (6) size and shape of thenar and palmar tubercles; (7) extent of toe webbing; (8) presence of outer metatarsal tubercle; (9) relative size of inner metatarsal tubercle; (10) ventral colouration; (11) presence of light line on dorsal face of tibia; (12) presence of light band on dorsum; (13) colour of lateral dorsal fold); (14) colour pattern on postaxial side of femur. The webbing formulae are given as proposed by Myers & Duellman (1982). Terminology for dermal dorsal ridges follows Perret (1979).

Bioacoustic sampling and call analyses

For the quantitative analysis of advertisement call features we recorded series of 2–10 advertisement calls (median: 10) given by a total of 45 individuals which called without acoustic interference of other individuals, using a Sony PCM–D50 Linear PCM Recorder with stereo microphones, Sony Deutschland GmbH, Cologne. Air temperature (to the nearest 0.1 ◦C) was registered at the calling sites (usually at 0.5–1.0m height within vegetation) immediately after recording using a TinyTag temperature logger, Gemini Data Loggers Ltd., UK. Recording dates, temperature range and number of individuals per taxon and site were: Butare, 5/3/2009, 7–18/10/2009, 5–11/9/2010 and 11/3/2011, 15.7–22.0 °C, n=4 *Ptychadena anchietae*/15 *Ptychadena nilotica*/18 *P. porosissima*; Mwogo, 2/10/2010 and 14/3/2011, 19.8–21.6 °C, n = 0/2/1; Gitarama, 3/10/2010, 21.1 °C, n = 1/2/2.

In addition to the analysis of the advertisement calls, we collected five advertising males per species in Butare and stimulated individuals to give release and/or distress calls by holding them between thumb and forefinger and gently compressing the sides (artificial amplexus; di Tada *et al.*, 2001). Slight pressure was necessary to elicit a series of release calls, whereas stronger pressure caused some individuals to give distress calls. The duration of amplexus simulation ranged between 30 s and 120 s per individual. Calls were recorded during the day at 17.0–21.5 ◦C air temperature. We analysed 20 release calls given by two *P. nilotica* and 43 by five *P. porosissima*, and seven distress calls given by one *P. anchietae*, a single one by *P. nilotica*, and 14 by three *P. porosissima*.

The vocalization produced during a single expiration is regarded a call. Calls are composed of

notes, and notes are either composed of a number of pulses or unpulsed. Repetitions of calls are regarded a call series.

We analysed the spectral features and the temporal structure of vocalisations using ADOBE Audition 3.0. Stereo recordings were converted to mono using a sampling rate of 44.1 kHz and resolution of 16 bits. Sonagrams and frequency analyses were prepared applying Blackman-Harris Fast Fourier transformation with a FFT size of 1024 Hz. Each advertisement and release call series was characterized by nine parameters: (1) call duration [ms]; (2) intercall interval [ms]; (3) call repetition rate [N/min]; (4) number of pulses per call [N]; (5) pulse duration [ms]; (6) interpulse interval [ms]; (7) pulse rate [N/s]; (8) fundamental frequency of complete call [Hz]; (9) dominant frequency of complete call [Hz]. Distress calls were characterized by four parameters: (1) call duration [ms]; (2) fundamental frequency [Hz]; (3) dominant frequency [Hz]; (4) number of discernible harmonics in the frequency range of 0–21 kHz. Advertisement, release and distress calls of each individual were described as the arithmetic means of usually 10 observations per call parameter to avoid pseudoreplication. Thus, degrees of freedom in statistical analyses refer generally to the number of individuals recorded not the total number of calls analysed.

At all investigated localities we conducted bioacoustic surveys and recorded the chorus of frogs at three distinct sites for five consecutive minutes. The recordings were scanned for *Ptychadena* advertisement calls to identify local species composition. Original recordings of all three species are deposited in Fonoteca Zoológica (Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, Madrid, Spain; http://www.fonozoo.com).

DNA barcoding and phylogenetic analyses

Liver tissue samples were taken from the specimens and stored separately in 98% ethanol. Samples from 13 specimens (Appendix 1) were used to sequence a fragment of the 16S mitochondrial rRNA gene, a suggested universal marker to barcode amphibians for species allocation (Vences *et al.* 2005). DNA was extracted 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 16S mitochondrial rRNA using standard primers (Palumbi *et al.* 2002) and standard PCR conditions (Palumbi 1996) with the following thermal cycle profile: 120 s at 94 ◦C, followed

by 33 cycles of 94 ◦C for 30 s, 53 ◦C 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 Highpure PCR Product Purification Kit (Roche Diagnostics). Sequencing was performed with the DYEnamic ET Terminator Cycle Sequencing Premix kit (GE Healthcare, Munich, Germany) for sequencing reactions run on a MegaBACE 1000 automated sequencer (GE Healthcare). DNA sequences were corrected and aligned by eye. The obtained sequences were compared with those in GenBank using a standard nucleotide-nucleotide BLAST search (Benson *et al.* 2004). See Appendix 2 for a complete list of sequences, collection sites and voucher specimens. Editing and analyses of pairwise distances were completed in MEGA5 (Tamura *et al.* 2011). Sequences were trimmed to the same length. The final alignment consisted of 548 base pairs. Calculations of pairwise distances and phylogenetic analyses (Maximum Likelihood and Maximum Parsimony) were carried out inMEGA5. For the Maximum Likelihood analysis, the Tamura-Nei substitution model and the heuristic Nearest-Neighbour-Interchange method were used. 5000 bootstrap replicates were run on each analysis.

Statistical analyses

Descriptive statistics depended on the outcome of the test for normality. Normally distributed data were described by the arithmetic mean and corresponding standard error and/or range, those deviating significantly by median and range. A principal component analysis was run on the morphometric data set including 18 variables with 59 observations (= individuals), including 58 specimens from Rwanda and a topotypic specimen of *P. nilotica* from Egypt. We compared the scores obtained for the principal components 2 and 3 describing shape to distinguish morphotypes without a priori assignment to taxa. The morphometric distances were adjusted for SVL by calculating a linear regression of each variable against SVL and storing the residuals as representatives of size-independent shape variables. This transformed data set was used for a discriminant analysis with taxa as predefined groups to optimize distinction among morphotypes and to remove morphometric variables which did not contribute significantly to the separation of morphotypes (removal criterion: F = 4.0).

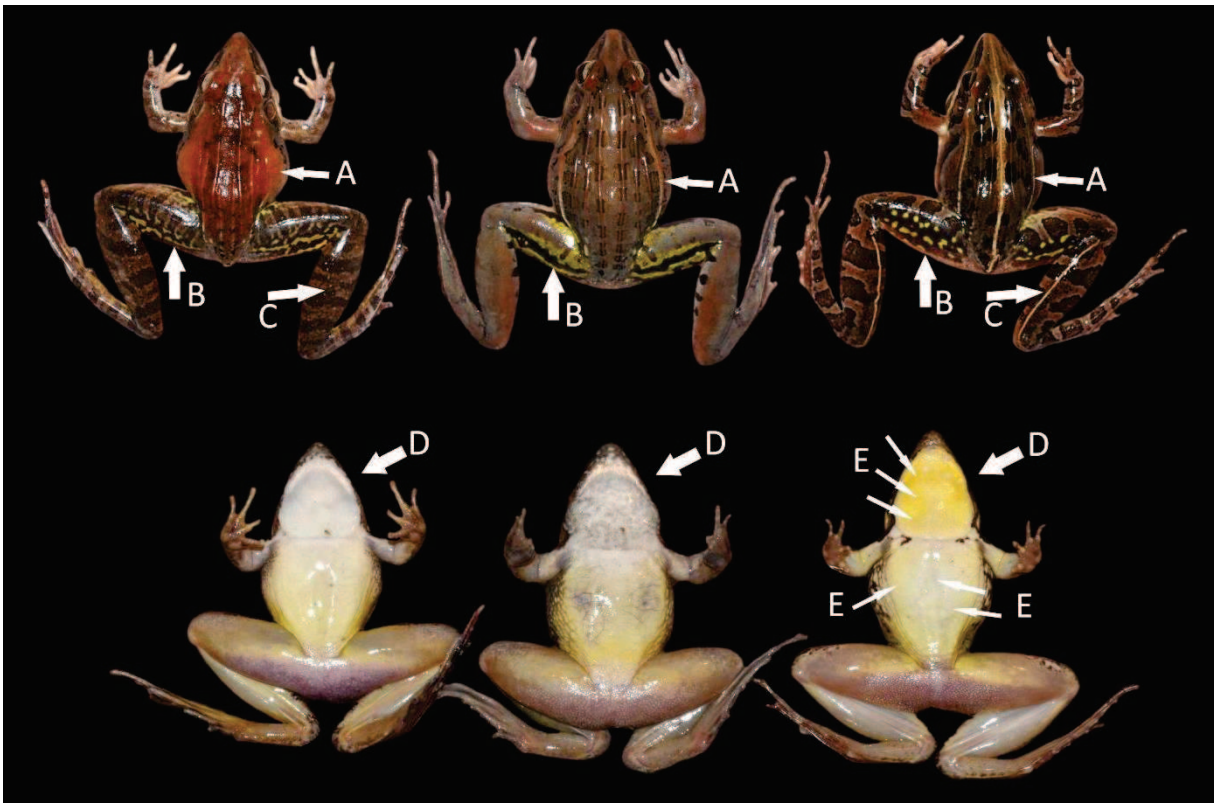


Figure 6. Dorsal view (top row) and ventral view (bottom row) of males of *Ptychadena anchietae* (left), *P. nilotica* (middle), and *P. porosissima* (right) from Rwanda. Arrows indicate distinguishing qualitative characters: (A) presence of light, prominent dorsolateral fold; (B) colour pattern on postaxial side of femur; (C) presence of light tibial line; (D) ventral colouration of head; (E) presence of small spiny tubercles on venter. See also Table 5.

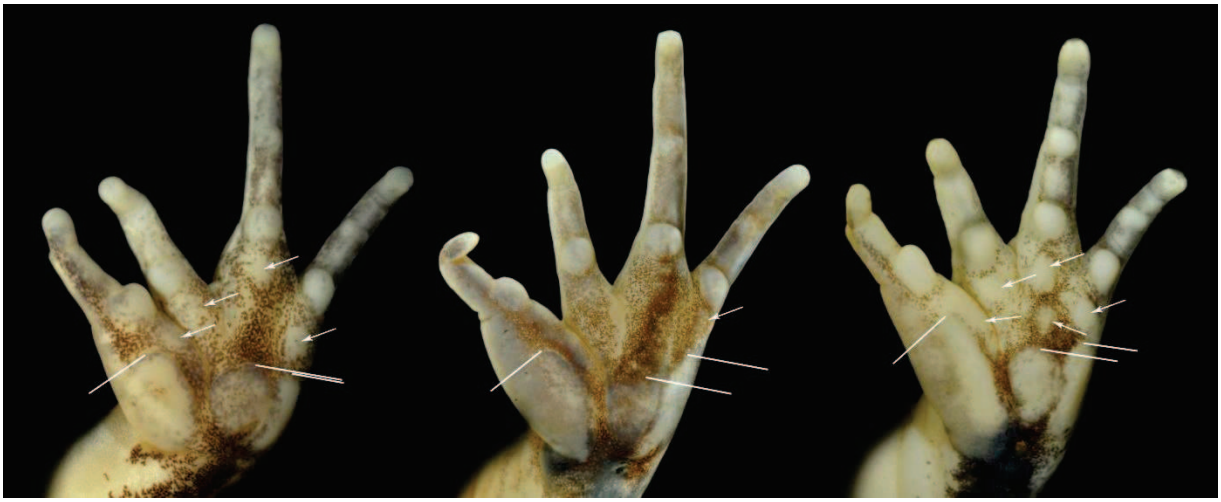


Figure 7. Volar view of hands of males of *Ptychadena anchietae* (left), *P. nilotica* (middle), and *P. porosissima* (right) from Rwanda. Arrows indicate the number of supernumerary metacarpal tubercles, lines indicate relative sizes of thenar tubercle and inner and outer palmar tubercle. See also Table 5.

Average advertisement call variables per individual were adjusted for temperature using the residuals of the corresponding linear regressions on air temperature. A discriminant analysis was run on the residual data matrix containing nine call variables and 45 observations (= individuals) to quantify the acoustic differentiation post hoc among the species. The most parsimonious variable set, in which those variables were removed which did not contribute significantly to the separation of groups (removal criterion: F = 4.0), was chosen using the backward selection procedure. Significance level was set at alpha = 0.05. All calculations were based on the procedures of the programme package STATGRAPHICS centurion for Windows, version XV.

Results

Using independent evidence from the morphological, bioacoustic and genetic data sets, we identified three species of *Ptychadena* inhabiting the surveyed localities (Figures 6–8; Tables 5 & 6). Two of these could be assigned to the currently recognized taxa *P. anchietae* (Bocage, 1868) and *P. porosissima* (Steindachner, 1867). The third taxon is a species of the *Ptychadena mascareniensis* (Duméril & Bibron, 1841) complex. The species differs strongly genetically from topotypic *P. mascareniensis*, and from clades referred to as *P.* cf. *mascareniensis* from Western and Central Africa and is more closely related to *Ptychadena newtoni* (Bocage, 1886) (see below). The name *Rana nilotica* Seetzen, 1855 is available for this species. We hereby resurrect this name. A detailed description of a topotypic specimen (ZFMK 77757), which was collected at the type locality in Cairo, Egypt, and from which genetic information is available, is in Appendix 3.

At seven of the nine investigated localities, all three species were present. *P. anchietae* was the only species present at the Rukarara site but was absent from the Akagera site where only *P. nilotica* and *P. porosissima* were recorded.

Morphological differentiation

The morphometric features of the three species are summarized in Table 6. Principal component analysis demonstrated that PC 2 unequivocally distinguished *P. nilotica* from the other two taxa, whereas the shape of *P. anchietae* did not differ significantly from that of *P.*

Table 5. Distinguishing qualitative characters of three *Ptychadena* species from Rwanda.

species	*P. anchietae*	*P. nilotica*	*P. porosissima*
relative length of Toes III and V	tips reaching to knee or slightly beyond, distal subarticular tubercle never reaching knee	tips reaching beyond knee, distal subarticular tubercle reaching knee or beyond	tips reaching to knee or slightly beyond, distal subarticular tubercle never reaching knee
position of vocal sac aperture	inferior, at ventral edge of arm insertion	superior, above dorsal edge of arm insertion	inferior, at ventral edge of arm insertion
spiny tubercles on venter	absent	absent	present in males
outer metatarsal tubercle	very faintly visible	distinctly present, rarely faintly visible	faintly visible, rarely distinct
inner metatarsal tubercle size	about half the length of metatarsus of Toe I	less than half the length of metatarsus of Toe I	more than half the length of metatarsus of Toe I
supernumerary metacarpal tubercles	one below each finger	only one below Finger IV, often indistinct	one below Fingers I, II, and IV; two, rarely one below Finger III
palmar and thenar tubercles	inner and outer palmar tubercle more or less equal in length; thenar tubercle oval, slightly longer than palmar tubercles	outer palmar tubercle longer than inner; thenar tubercle elongate, about as long as outer palmar tubercle	outer palmar tubercle longer than inner; thenar tubercle elongate, longer than outer palmar tubercle
toe webbing	I0.5-2II0.5-2III(0.5-1)-2IV2-0.5V	I(1.5-1.75)-(2-2.25)II1.5-(2.75-3)III(1.75-2)-3IV2.75-(1-1.5)V	I(1.75-2)-2.25II1.5-3III1.75-(3-3.25)IV3-(1-1.5)V
ventral colouration	head white, trunk yellow	head white, mottled with grey; trunk yellow	head and trunk yellow
light tibial line	absent	present or absent	present
light dorsal band	absent	present or absent	present or absent
light, prominent dorsolateral fold	usually absent	present	present
Colour pattern on postaxial side of femur	irregularly delimited, reticulated, longitudinal bands, alternately yellow and dark brown coloured	relatively sharply delimited longitudinal bands, alternately yellow and black coloured	yellow spots diffusely arranged in longitudinal rows on a dark brown background

Table 6. Morphometric features of three *Ptychadena* species from Rwanda. Data are given as arithmetic means and minimum and maximum values (in mm). Hyphenated letters indicate significant difference among species (P < 0.05, multiple range test with Bonferroni correction).

Morphometric character [mm]	*P. anchietae*		*P. nilotica*	
	Males N = 12	Females N = 3	Males N = 13	Females N = 10
Snout-vent length	40.2[a] (38.0–42.4)	49.0[b] (46.7–51.3)	42.0[a] (37.2–45.2)	49.1[b] (45.6–53.1)
Hindlimb length	79.9[a] (74.2–84.9)	98.9[c] (96.0–101.2)	77.2[a] (70.1–85.2)	89.6[b,c] (78.0–103.9)
Femur length	23.0[a,b] (21.9–24.5)	29.0[d] (28.3–29.6)	21.5[a] (19.4–24.0)	25.5[c,d] (23.0–28.5)
Tibiofibula length	26.3 [b,c] (24.4–28.0)	33.1[e] (31.9–33.8)	23.4[a] (21.1–26.1)	27.5[d] (23.6–32.1)
Tarsus length	34.5[a] (31.6–36.5)	42.7[b] (40.6–44.1)	36.2[a] (32.4–40.1)	42.8[b] (35.9–49.6)
Foot length	24.8[a] (22.4–26.1)	30.5[c] (29.1–31.3)	25.7[a,b] (22.8–28.2)	29.3[c] (25.6–33.9)
Forelimb length	16.9[a] (15.9–18.1)	21.0[c] (20.0–21.7)	17.7[a,b] (16.0–19.3)	20.5[c] (17.9–23.7)
Hand length	10.0[a,b] (9.6–10.7)	12.3[c] (12.1–12.6)	10.4[a,b] (9.5–11.6)	11.9[c] (10.6–13.8)
Head width	13.8[a] (12.5–15.4)	16.7[c] (16.4–17.0)	13.8[a,b] (12.4–15.9)	16.4[c] (14.2–18.8)
Head length	15.5[a] (14.1–17.6)	18.7[b] (18.2–19.1)	16.2[a] (14.7–18.4)	18.3[b] (16.6–20.4)
Interorbital distance	2.7[b,c] (2.4–3.1)	3.1[b,c] (3.0–3.2)	2.2[a] (1.9–2.5)	2.5[b] (2.2–2.7)
Eyelid width	2.8[a] (2.5–3.1)	3.2[a,b] (3.0–3.3)	2.7[a] (2.3–3.2)	3.0[a,b] (2.4–3.5)
Eye diameter	4.3[a] (3.6–4.9)	5.2[b] (5.0–5.5)	4.5[a] (4.1–5.1)	5.1[b] (4.8–5.5)
Typanum diameter	3.2[a] (2.9–3.6)	4.1[b,c] (3.7–4.6)	3.7[b] (3.3–4.1)	4.1[c] (3.7–4.7)
Eye–nostril distance	4.0[b] (3.7–4.3)	5.2[c] (4.9–5.7)	3.6[a] (3.4–4.0)	4.2[b] (3.7–4.9)
Snout–nostril distance	3.5[a] (2.9–4.0)	4.4[b] (4.3–4.5)	3.4[a] (2.9–3.8)	3.7[a,b] (3.3–4.1)
Internarial distance	3.8[b] (3.5–4.1)	4.8[c] (4.7–4.9)	3.4[a] (3.0–3.6)	3.7[a,b] (2.0–4.5)
Snout length	7.4[a,b] (6.6–8.1)	9.5[c] (9.0–10.1)	7.0[a] (6.3–7.7)	7.9[b] (7.1–9.3)

Morphometric character [mm]	*P. porosissima*	
	Males N = 11	Females N = 9
Snout-vent length	40.8[a] (37.3–44.1)	46.4[b] (39.0–52.1)
Hindlimb length	78.0[a] (73.0–84.4)	88.3[b] (74.3–94.1)
Femur length	21.9[a] (20.2–24.1)	24.4[b,c] (19.8–27.6)
Tibiofibula length	24.8[a,b] (23.3–26.5)	28.7[d] (25.2–31.0)
Tarsus length	35.5[a] (33.3–38.8)	40.0[b] (32.6–43.5)
Foot length	24.4[a] (23.0–26.7)	27.4[b,c] (23.1–29.3)
Forelimb length	17.2[a] (16.0–18.6)	18.7[b] (16.1–20.9)
Hand length	9.7[a] (8.9–10.8)	10.7[b] (9.1–11.7)
Head width	14.1[a,b] (13.1–15.0)	15.1[b,c] (12.5–17.1)
Head length	15.5[a] (14.2–17.8)	17.1[a,b] (13.6–19.1)
Interorbital distance	2.6[b] (2.3–2.7)	3.1[c] (2.8–3.6)
Eyelid width	2.8[a] (2.3–3.1)	3.1[b] (2.6–3.5)
Eye diameter	4.2[a] (3.9–4.5)	4.6[a] (3.9–5.1)
Typanum diameter	3.1[a] (2.9–3.49	3.6[b] (3.3–3.9)
Eye–nostril distance	3.5[a] (3.1–3.9)	4.2[b] (3.6–4.7)
Snout–nostril distance	3.4[a] (2.9–3.7)	4.1[b] (2.8–4.7)
Internarial distance	3.6[a,b] (3.4–4.0)	4.3[c] (3.7–4.7)
Snout length	7.0[a] (6.3–7.8)	8.1[b] (6.2–10.1)

porosissima (Figure 9a). Discriminant analysis based on the residuals of SVL-adjusted morphometric variables yielded one most parsimonious model which combined six variables and had a classification success of 98.3% among the three taxa (Table 7; Figure 9b). In addition, the three species can be distinguished unequivocally by a number of qualitative characters which are summarized in Table 5.

Figure 8. Plantar view of feet (left) and length of Toe V in relation to tibia (right) of *Ptychadena anchietae* (top), *P. nilotica* (middle), and *P. porosissima* (bottom). Lines on the left indicate the extent of toe webbing and the relative size of the inner metatarsal tubercle ("="): tubercle half the length of metatarsus, "<": tubercle less than half the length of metatarsus, ">": tubercle more than half the length of metatarsus). Arrows on the right indicate the position of the distal subarticular tubercle of Toe V. See also Table 5.

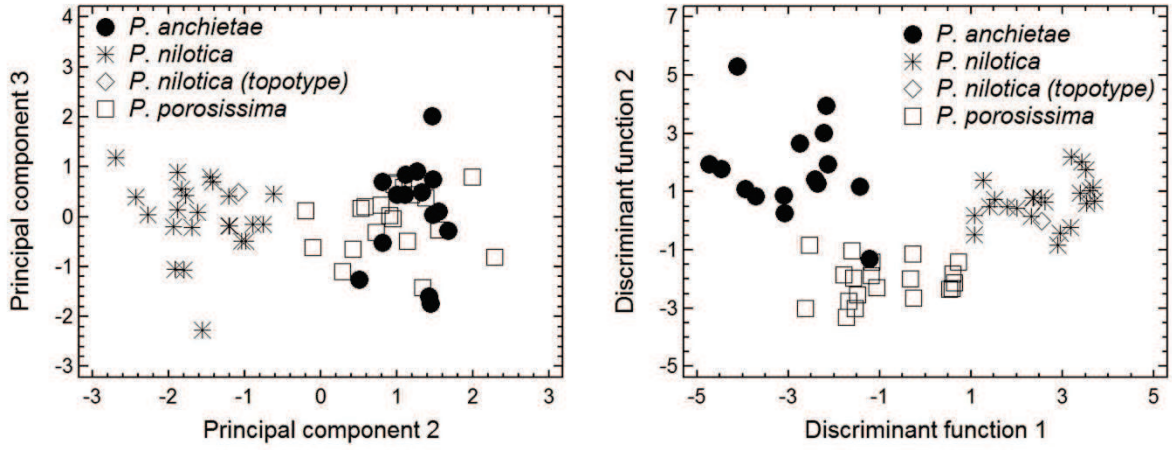


Figure 9. Morphological differentiation among 58 *Ptychadena* individuals collected in Rwanda and a topotypic specimen of *P. nilotica* from Cairo, Egypt. (A) Individual scores obtained for PC 1 (eigenvalue: 12.3, variance explained: 72.6%, PC 2 (2.0, 11.5%), and PC 3 (0.6, 3.6%), respectively. (B) Individual discriminant scores obtained for 15 individuals of *P. anchietae*, 24 individuals of *P. nilotica*, and 20 individuals of *P. porosissima*. Statistical details are given in Table 7.

The specific ranges of most classic morphometric ratios overlapped widely and did not differ significantly (ANOVA, $p > 0.05$). The exceptions were (ANOVA, $p < 0.05$): The TFL/SVL ratio separated P. nilotica ($\bar{x}_{males}$ = 0.56, range 0.50–0.60; $\bar{x}_{females}$ = 0.56, 0.54–0.60) from *P. anchietae* ($\bar{x}_{males}$ = 0.66, 0.61–0.68; $\bar{x}_{females}$ = 0.68, 0.65–0.72) with *P. porosissima* being intermediate ($\bar{x}_{males}$ = 0.61, 0.58–0.65; $\bar{x}_{females}$ = 0.62, 0.56–0.66). By the FOT/TFL ratio, *P. nilotica* ($\bar{x}_{males}$ = 1.10, 1.04–1.12; $\bar{x}_{females}$ = 1.06, 1.03–1.10) was distinguishable from *P. anchietae* ($\bar{x}_{males}$ = 0.94, 0.85–1.00; $\bar{x}_{females}$ = 0.92, 0.91–0.93) and *P. porosissima* ($\bar{x}_{males}$ = 0.98, 0.94–1.01; $\bar{x}_{females}$ = 0.96, 0.92–1.01). None of the morphometric ratios differed significantly between *P. anchietae* and *P. porosissima*.

Table 7. Most parsimonious discriminant functions (procedure: backward selection) based on six SVL-adjusted morphometric features (residuals) to distinguish among three *Ptychadena* species from Rwanda.

(A) Statistical significance:

Discriminant function	Eigen-value	Relative percentage	Canonical correlation	Wilks Lambda	Chi-squared	Degrees of freedom	Statistical significance
1	5.42	68.1	0.9189	0.0439	167.3	12	P < 0.0001
2	2.54	31.9	0.8474	0.2820	67.7	5	P < 0.0001

(B) Unstandardized coefficients of the discriminant functions:

call parameter (residuals)	discriminant function 1	discriminant function 2
Tympanum diameter	2.9299	0.6796
Tibiofibula length	-0.6074	-0.14482
Foot length	-0.0242	1.5489
Tarsus length	0.3610	-1.1521
Interorbital distance	-1.9072	-2.2738
Eye-to-nostril distance	-2.0897	4.4712
Constant	6.7743E-9	-6.7401E-7

(C) Classification success:

predicted species actual species	*P. anchietae*	*P. nilotica*	*P. porosissima*
P. anchietae	14 (93.3%)	0	1 (6.7%)
P. nilotica	0	23 (100%)	0
P. porosissima	0	0	20 (100%)

Vocal repertoire

We recorded three types of vocalisations, the spontaneously given advertisement call and release and distress calls after stimulation (Figures 10–12). Advertisement and release calls consisted of a single pulse group with regular amplitude modulation, whereas distress calls were mainly tonal and ended in a few pulses. Dominant frequency of advertisement calls was either the second or the fourth harmonic of the fundamental frequency, whereas that of release calls was mostly identical with the fundamental frequency. Quantitative call features are summarized in Table 8.

Ptychadena males called mostly at the shore region of small permanent water bodies or in flooded crop fields. The advertisement calls differed among the three species with respect to all call variables, but only fundamental and dominant frequency yielded a clear univariate distinction (Table 9; ANCOVA with Multiple Range Test, $P < 0.05$). Biplots of dominant frequency, pulse rate, pulse duration and interpulse interval permitted the acoustic identification of each species on a single call basis (Figure 13). Discriminant analysis based on the residuals of temperature-adjusted call variables revealed that the most parsimonious model yielding a significant separation of the three taxa included three parameters, call duration, interpulse interval and pulse rate (Table 9). Except for a single *P. porosissima* male, all individuals were assigned to the correct taxon (average classification success: 97.8 %; Figure 14).

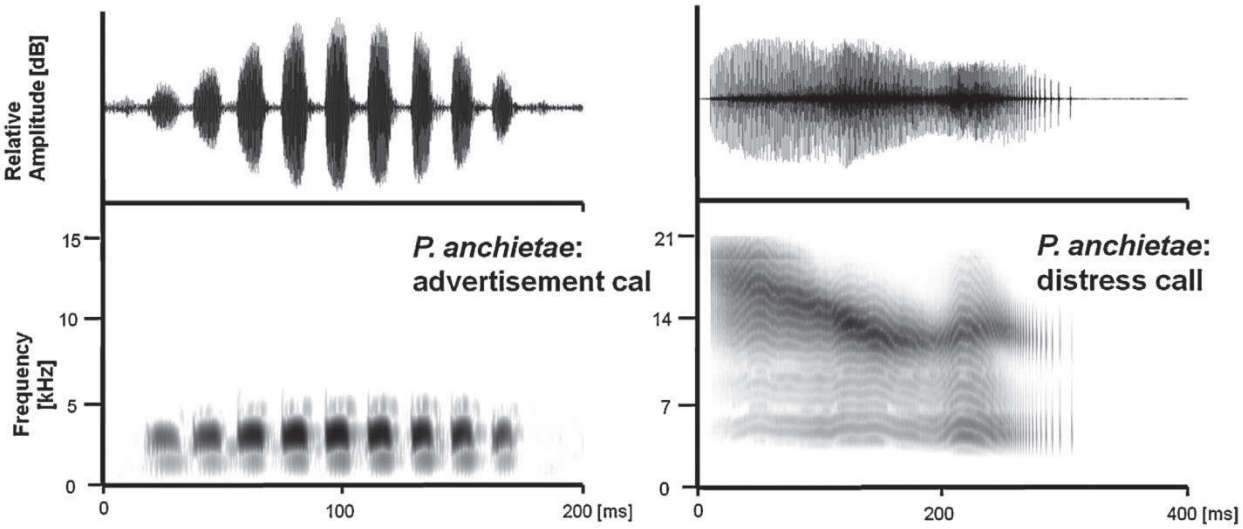
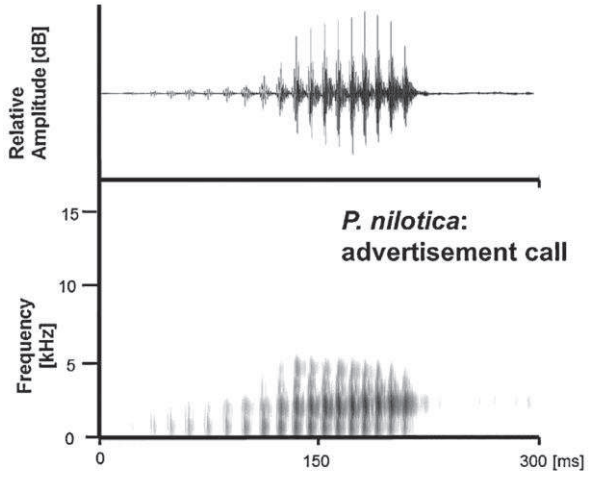
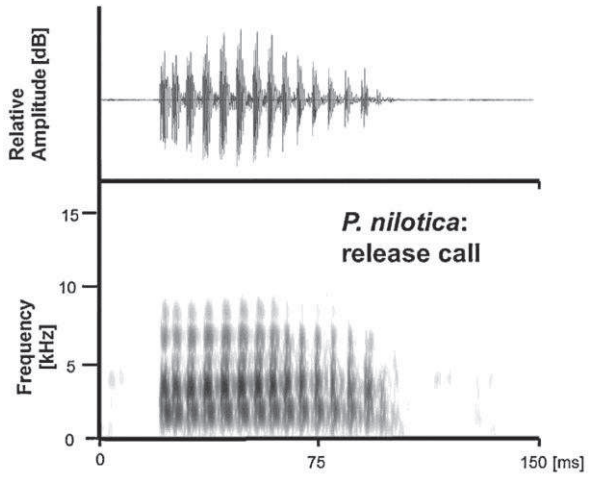


Figure 10. Vocal repertoire of *Ptychadena anchietae*. Advertisement call (Mwogo, 19.8 ◦C; top) and distress call (Butare, 20.0 ◦C; bottom) are presented as oscillogram and corresponding sonagram.





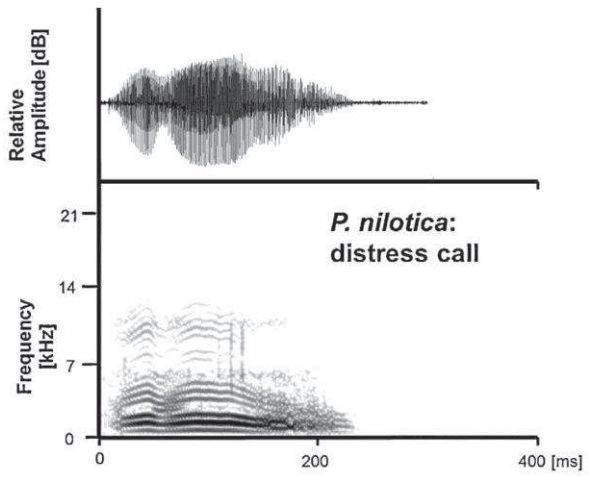
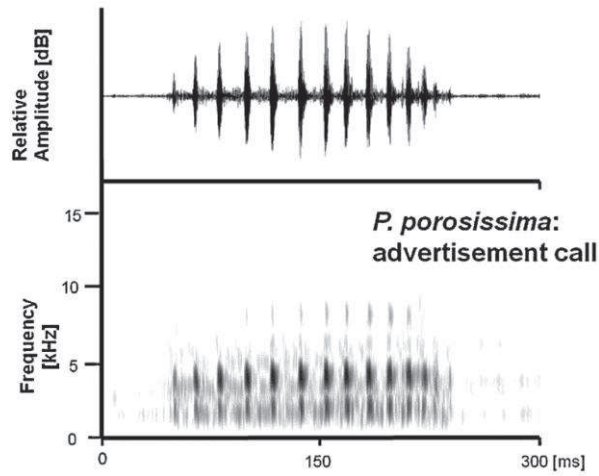
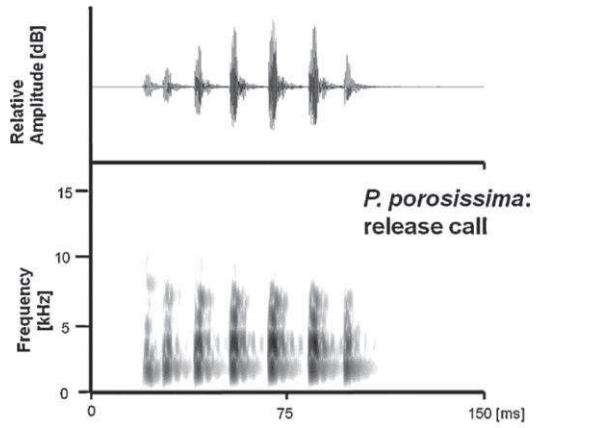


Figure 11. Vocal repertoire of *Ptychadena nilotica*. Advertisement call (Butare, 16.6 °C; top), release call (Butare, 17.0 °C; centre) and distress call (Cyangugu, 21.0 °C; bottom) are presented as oscillogram and corresponding sonagram.





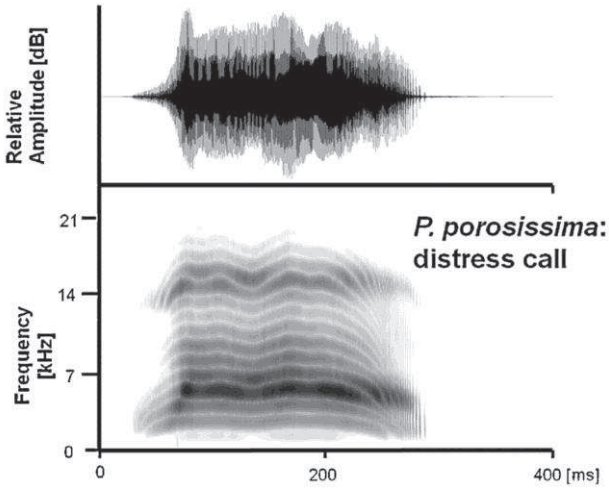


Figure 12. Vocal repertoire of *Ptychadena porosissima*. Advertisement call (Mwogo, 21.6 °C; top), release call (Butare, 17.0 °C; centre) and distress call (Butare, 20.0°C; bottom) are presented as oscillogram and corresponding sonagram.

Table 8. Features of the advertisement, release and distress calls of three *Ptychadena* species from Rwanda. Data are given as temperature-adjusted least square means and corresponding 95% confidence interval. Hyphenated letters indicate significant difference among advertisement call features ($P < 0.05$, Multiple range test with Bonferroni correction). Due to the low number of individuals given release or distress calls we refrained from statistical comparison between species.

Call parameter	Advertisement call		
	P. anchietae $N = 19$	*P.* nilotica $N = 5$	*P. porosissima* $N = 21$
Call duration [ms]	205^{b} (192–219)	161^{a} (134–187)	191^{ab} (178–204)
Intercall interval [ms]	865^{b} (710–1019)	843^{ab} (539–1146)	456^{a} (305–606)
Call repetition rate [N/min]	62^{a} (51–72)	88^{ab} (67–110)	101^{b} (90–111)
Notes per call [N]	1	1	1
Pulses per note [N]	11^{a} (10–12)	16^{b} (14–18)	10^{a} (9–11)
Pulse duration [ms]	10.6^{b} (9.9–11.4)	4.8^{a} (3.4–6.3)	6.1^{a} (5.4–6.9)
Interpulse interval [ms]	5.5^{a} (4.6–6.4)	4.3^{a} (2.5–6.1)	13.2^{b} (12.3–14.1)
Pulse rate [N/s]	54^{a} (51–56)	97^{b} (92–102)	52^{a} (50–55)
Fundamental frequency [Hz]	1600^{b} (1563–1636)	619^{a} (575–662)	$1\,952^{c}$ (1908–1995)
Dominant frequency [Hz]	3322^{b} (3239–3404)	$2\,434^{a}$ (2272–2597)	$4\,036^{c}$ (3952–4120)
Harmonics [*N*/21 kHz]	–	–	–

Call parameter	Release call		Distress call		
	P. nilotica $N = 2$	*P. porosissima* $N = 5$	*P. anchietae* $N = 1$	*P.* nilotica $N = 1$	*P. porosissima* $N = 3$
Call duration [ms]	63 (56–70)	82 (78–87)	332	220	241 (0–702)
Intercall interval [ms]	499 (139–858)	787 (560–1014)	–	–	–
Call repetition rate [N/min]	118 (80–156)	100 (76–124)	–	–	–
Notes per call [N]	1	1	1	1	1
Pulses per note [N]	13 (11–15)	7 (6–8)	–	–	–
Pulse duration [ms]	2.1 (1.5–2.7)	2.9 (2.5–3.2)	–	–	–
Interpulse interval [ms]	2.1 (0–4.8)	11.8 (10.1–13.5)	–	–	–
Pulse rate [N/s]	196 (173–219)	189 (174–203)	–	–	–
Fundamental frequency [Hz]	1 632 (1544–1718)	1 743 (1688–1798)	1 784	701	1 111 (652–1570)
Dominant frequency [Hz]	1 632 (1544–1718)	2 531 (1736–3326)	10 272	1 421	3 937 (659–7216)
Harmonics [*N*/21 kHz]	–	–	18	28	20 (12–27)

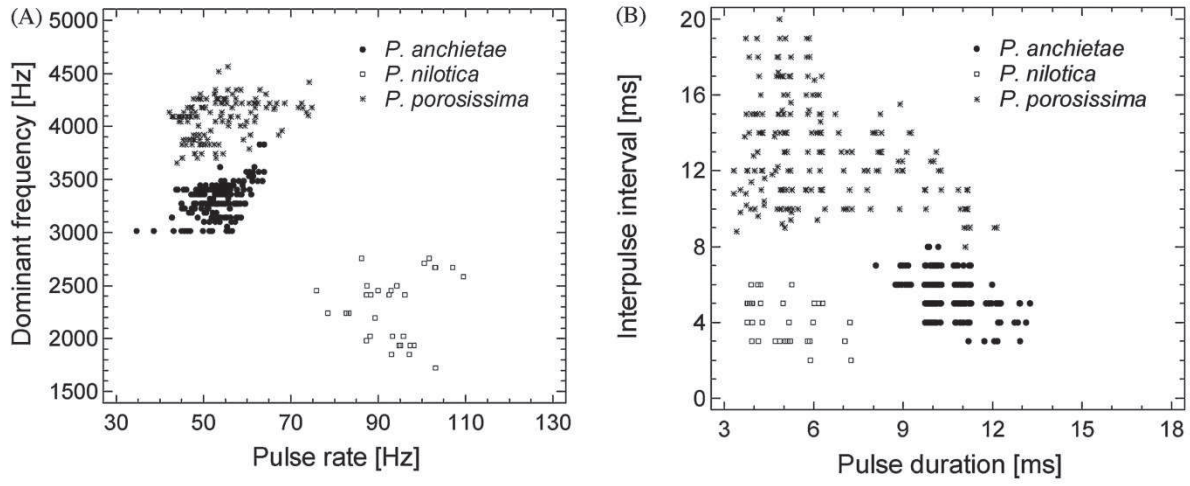


Figure 13. Features of 417 advertisement calls providing an unequivocal distinction among 19 specimens of *Ptychadena anchietae*, 5 specimens of *P. nilotica*, and 21 specimens of *P. porosissima*. (A) Pulse rate vs. dominant frequency; (B) Pulse duration vs. interpulse interval. Each symbol represents a single call.

Table 9. Discriminant functions based on three temperature-adjusted advertisement call parameters (residuals) to distinguish among the *Ptychadena* spp.

(A) Statistical significance:

Discriminant function	Eigen-value	Relative percentage	Canonical correlation	Wilks Lambda	Chi-squared	Degrees of freedom	Statistical significance
1	7.08	76.1	0.9360	0.0384	133.7	6	P << 0.0001
2	2.23	23.9	0.8308	0.3098	48.0	2	P << 0.0001

(B) Unstandardized coefficients of the discriminant functions:

call parameter (residuals)	discriminant function 1	discriminant function 2
Call duration	0.0206	-0.0052
Interpulse interval	0.3735	-0.4196
Pulse rate	0.2315	0.0027
Constant	-5.056E-8	-7.626E-8

(C) Classification success:

predicted species / actual species	*P. anchietae*	*P. nilotica*	*P. porosissima*
P. anchietae	19 (100%)	0	0
P. nilotica	0	5 (100%)	0
P. porosissima	1 (4.8%)	0	20 (95.2%)

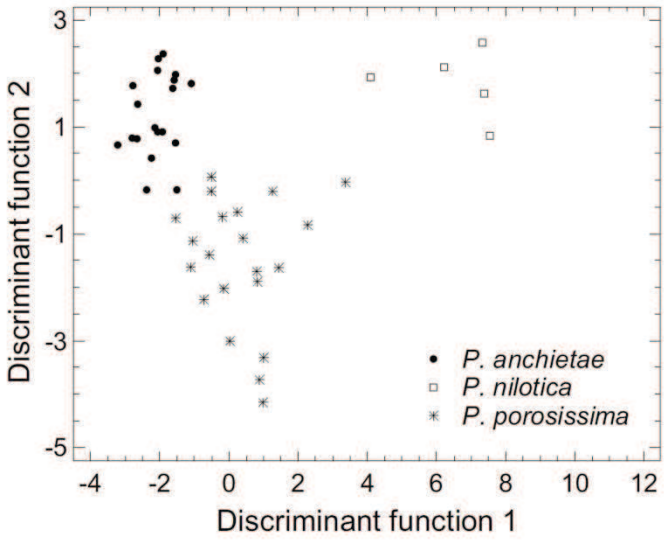


Figure 14. Discriminant analysis based on eight temperature-adjusted advertisement call variables to distinguish among 19 specimens of *Ptychadena anchietae*, 5 specimens of *P. nilotica*, and 21 specimens of *P. porosissima*. Each symbol represents the average advertisement call features of an individual.

Exposed to artificial amplexus, two *P. nilotica* and five *P. porosissima* emitted release calls, whereas none of the tested *P. anchietae* responded. Call structure resembled the conspecific advertisement call superficially, but calls were shorter, pulse rate higher, and dominant frequency lower (Table 8). The low number of individuals producing release calls did not allow statistical comparisons, but available data suggest a species-specific differentiation analogous to that of advertisement calls. One *P. anchietae*, one *P. nilotica* and three *P. porosissima* males felt disturbed enough by the handling to emit single distress calls which did not resemble advertisement nor release calls (Table 8). Common features were frequency modulation, dominant frequency two to five times greater than fundamental frequency and many harmonics reaching up to at least 21 kHz. The calls were emitted with the mouth open.

Genetic analysis

Comparison of the mitochondrial 16S rRNA gene sequences confirmed the distinctness of the three species and showed that they were genetically comparatively widely separated (uncorrected p-distances: 10.6–14.3%). Comparison with sequences stored in GenBank showed that specimens of *P. anchietae* from Rwanda are almost identical genetically (p-distance 0.2–0.4 % in the 16S sequence) to specimens from Uganda (Semliki National Park) and Kenya (Kakamega Forest) but differ slightly to moderately from specimens from Somalia (Karin: 1.2–1.4%), Kenya (Runda: 0.9 %, Kararacha Pond: 1.4%), Tanzania (Makuyuni: 1.7 %), and South Africa (Mtunzoni, St. Lucia: 2.1 %). The 16S sequences of specimens of *P. nilotica* from Rwanda are identical to almost identical (uncorrected p-distances 0.0–0.4%) to sequences from Kenya (Mount Kenya, Taita Hills, Nakuru), Egypt, and Uganda (Semliki NP, Lake Victoria) and differ moderately from sequences from southern Tanzania (1.9–2.4%), of which all were assigned to "*mascareniensis* Clade A" by Vences *et al.* (2004). The sequences of *P. nilotica* differ strongly from sequences from the type locality of *P. mascareniensis*, Reunion, Mauritius and Madagascar (*mascareniensis* s. s.; 5.8–7.2%), from Benin (*mascareniensis* Clade B of Vences *et al.* 2004; 5.9–7.2%), Ivory Coast (*mascareniensis* Clade C of Vences *et al.* 2004; 5.3–6.0%), from Uganda (Kampala, Rwenzori Mts.) and Kenya (Kakamega Forest; *mascareniensis* Clade D of Vences *et al.* 2004; 5.7–6.1%), and from the Central African Republic (Dzanga-Sangha; *mascareniensis* Clade E of Measey *et al.* 2007; 5.7–6.4%). *P. nilotica* is more closely related to *Ptychadena newtoni* from Sao Tomé (p-distance

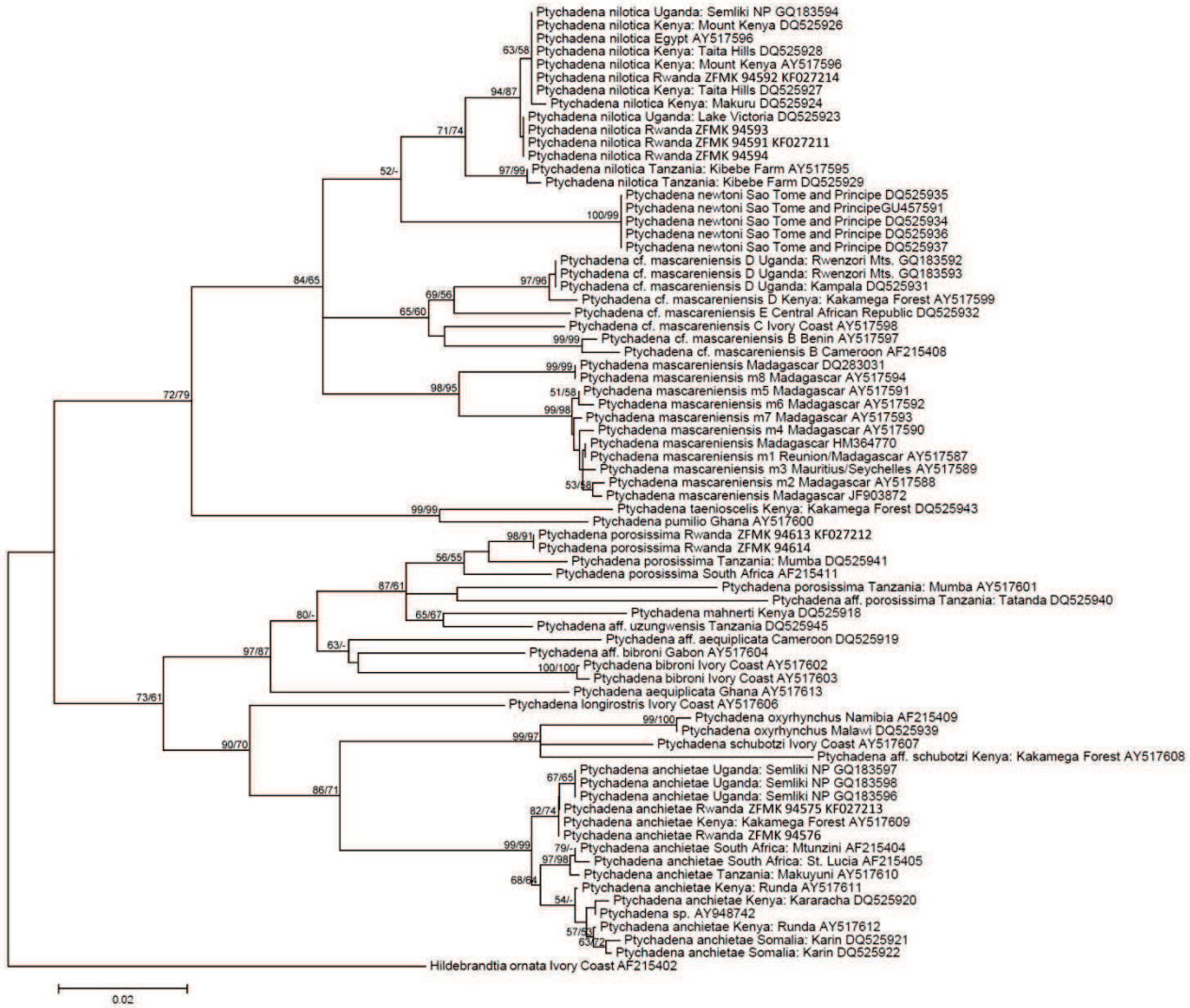


Figure 15. Maximum likelihood phylogram of species in the genus *Ptychadena* and *Hildebrandtia ornata* as outgroup, based on comparison of 548 base pairs of the mitochondrial 16S rRNA gene. Included are specimens from Rwanda and samples taken from GenBank (with accession numbers and origin). Numbers above nodes are percentage support values from maximum likelihood and maximum parsimony analyses. Only values above 50% are shown.

4.9–5.9 %) than to the other clades of *P. mascareniensis* s. l. (Figure 15). The sequence of specimens of *P. porosissima* from Rwanda differs considerably from those of specimens from South Africa (2.7%) and strongly from a sequence of specimen labelled "*P.* aff. *porosissima* A" from Tanzania (Tatanda: 5.6%). There are two sequences from Mumba, Tanzania, deposited in GenBank, from which the Rwandan sequences differ by 1.7 % (DQ525941) and 6.3 % (AY517601), although both sequences supposedly originate from the same specimen (AC 2122).

Discussion

We recorded three species in the wetlands of the upper Nile in Rwanda: *P. anchietae*, *P. nilotica*, and *P. porosissima*. Whereas *P. nilotica* and *P. porosissima* have been recorded from Rwanda before (e.g. Nieden 1913; Laurent 1954; Fischer & Hinkel 1992), the occurrence of *P. anchietae* was only suspected by Poynton *et al.* (2004) but there were no confirmed records (see also Spawls *et al.* 2006). The species are clearly differentiable by each of the three approaches (external morphology, bioacoustics, and molecular genetics).

Resurrection of Ptychadena nilotica *(Seetzen, 1855)*

The sequences of the 16S gene from Rwandan specimens are identical to almost identical to sequences from topotypic specimens from Cairo (Egypt), Kenya (Mount Kenya, Taita Hills, Nakuru), Uganda (Semliki NP, Lake Victoria), and differ moderately from sequences from southern Tanzania. In contrast, the 16S sequences of these populations differ strongly from sequences from the type locality of *P. mascareniensis* (p-distances 5.8–7.2%; Vences *et al.* 2004) and from clades referred to as *P.* cf. *mascareniensis* from Western and Central Africa, demonstrating that they are not conspecific and require a distinct taxonomic treatment. The oldest available name for these populations is *Rana nilotica* Seetzen, 1855. This name dates back to the description of three specimens from Cairo, Egypt, in Ulrich Jasper Seetzen's travel report which was published in 1855. The specimens were depicted in the "Description de l'Égypte", published by order of Napoleon Le Grand (1809; see also Peters 1863). Seetzen (1855) did not state whether he deposited the specimens in a scientific collection and their whereabouts are unclear, and therefore they are currently not available for examination. Frost (2011) suspects them in the collection of the ZMB, Berlin, but this is not true (F. Tillack, pers. comm.). Only one further species of *Ptychadena*, *P. schillukorum* (Werner, 1908), occurs in Egypt (Baha el Din 2005). The type specimens of *R. nilotica* depicted in the "Description de l'Égypte" show several characters including distinct dorsal dermal ridges, relatively sharply delimited alternately yellow and black coloured longitudinal bands on the postaxial side of the femur, and tips of Toes III and V considerably extending beyond knee when legs are folded, that readily distinguish them from *P. schillukorum* which has indistinct dorsal ridges, which are broken up into elongate disjunct warts, postaxial side of femur mottled with dark grey spots, and tips of Toes III and V reaching to knee only (Baha el Din

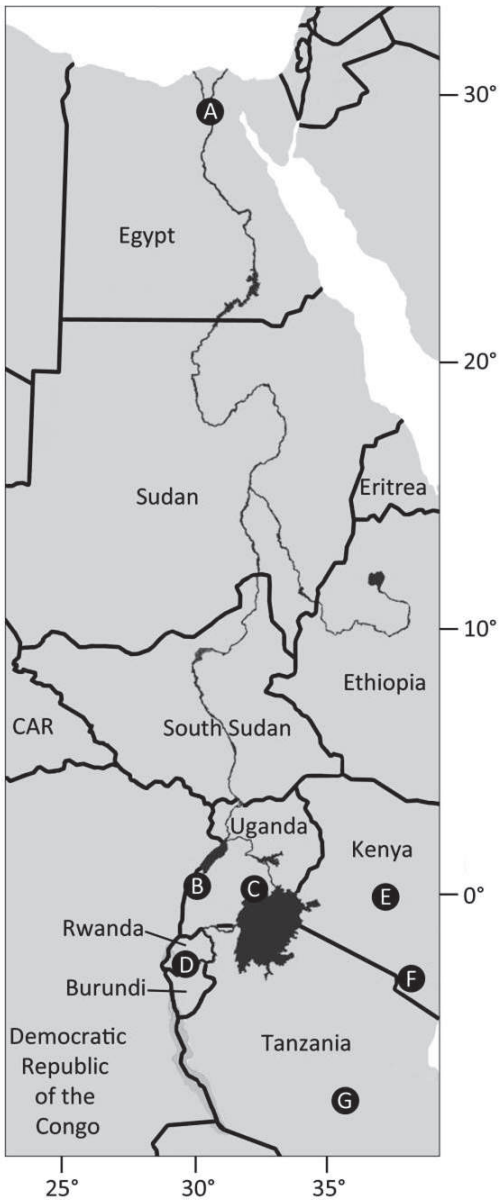


Figure 16. Map of northeastern Africa showing the course of the Nile River and the localities of confirmed records of *Ptychadena nilotica*: (A) type locality at Cairo, Egypt; (B) Semliki National Park, Uganda; (C) Lake Victoria, Uganda; (D) Upper Nile, Rwanda; (E) Mount Kenya, Kenya; (F) Nakuru, Kenya; (G) Taita Hills, Kenya; and (H) Kibebe Farm, Tanzania.

2005). We describe a topotypic specimen of *P. nilotica* from Cairo (ZFMK 77757) in detail in Appendix 3. The characters mentioned by Seetzen (1855) in the description match the ones of this specimen and of the specimens from Rwanda. The topotype clusters with the Rwandan specimens in both the principal component analysis and the discriminant analysis (Figure 9). Furthermore, Tawfik *et al.* (1994) provided morphometric character ratios of topotypic specimens from Cairo which are in agreement with those of Rwandan specimens

(values given as arithmetic means ± standard error and ranges for Rwandan specimens; SVL/TFL: Cairo 1.73 ± 0.025 / Rwanda 1.78 ± 0.017, 1.66–1.99; SVL/HW: Cairo 3.13 ± 0.104 / Rwanda 3.02 ± 0.032, 2.82–3.32; SVL/SL: Cairo 6.44 ± 0.156 / Rwanda 6.09 ± 0.066, 5.49–7.13; HW/SL 2.01 ± 0.047 / Rwanda 2.01 ± 0.022, 1.86–2.33; inner metatarsal one-third the length of Toe I). Calls of topotypic *P. nilotica* from Cairo (Akef & Schneider 1995) are indistinguishable from those recorded in Rwanda. Since all available evidence supports the validity of the taxon *P. nilotica* for Ridged Frogs which inhabit the Nile valley and its tributaries, we assign the populations formerly referred to as "*P.* cf. *mascareniensis* (Clade A)" (Vences *et al.* 2004; Measey *et al.* 2007) to *P. nilotica*. Figure 16 shows the localities of confirmed records of *Ptychadena nilotica*.

Morphological variation

The three species are similar in size and shape but using combinations of morphometric characters, they can be distinguished with both principal component analysis and discriminant analysis. The same applies to several qualitative characters which are of major importance for identification of specimens in the field. Examples are foot webbing, relative size of the inner metatarsal tubercle, colour pattern on the postaxial side of the thigh and in most specimens the number of supernumerary metacarpal tubercles (Table 5). By some characters only one species or sex can be distinguished from the other two, e.g. subarticular tubercles of Toes III and V reaching to knee (*P. nilotica*), large, spiny ventral tubercles present (males of *P. porosissima*), vocal sac aperture above dorsal edge of insertion of arms (males of *P. nilotica*), reduced number of supernumerary metacarpal tubercles (*P. nilotica*), ventral side of head and chest yellow (*P. porosissima*). The diagnostic values of presence and distinctness of the outer metatarsal tubercle are doubtful. This character is often used in species diagnoses and determination keys (e.g. Schmidt & Inger 1959; Poynton 1970; Channing & Howell 2006; du Preez & Carruthers 2009; Frétey *et al.* 2011), but in the specimens from Rwanda it was very variable. In the individuals examined, the tubercle development varied from a small, but traceable patch of callous tissue to a distinct, prominent tubercle. However, du Preez & Carruthers (2009) state that the tubercle is absent in the three studied species (referring, however, to populations from southern Africa, with the taxon they refer to as *P. mascareniensis* probably not being conspecific with *P. nilotica*). In some of Rwandan specimens the outer metatarsal tubercle was distinct on one foot but

only faintly visible on the other, similar to the situation in *Ptychadena schillokorum* observed by Poynton (1970; as *Ptychadena floweri* [Boulenger, 1917]). Thus, presence/absence data of the outer metatarsal tubercle should be used with caution for species diagnosis in *Ptychadena*.

Bioacoustic variation

Advertisement call differentiation corroborates morphological differentiation, as shown is many anuran species before (reviewed in Schneider & Sinsch 2007). The specific call features differ unequivocally among the three species (Table 8). Calls of topotypic *P. nilotica* from Cairo (Akef & Schneider 1995) are indistinguishable from those recorded in Rwanda. Advertisement calls of the three species recorded in Kenya and Uganda (Pickersgill 2007; Bwong *et al.* 2009), Tanzania (van den Elzen & Kreulen 1979; Pickersgill 2007), East Africa (Channing & Howell 2006), Malawi (Mercurio 2011), Mozambique and South Africa (Pickersgill 2007), and southern Africa (du Preez & Carruthers 2009) agree widely with those from Rwanda. Experienced researchers are able to tell the calls apart without sophisticated analyses. Consequently, bioacoustic surveys are most useful to detect and identify *Ptychadena* males in the field without need to collect specimens for morphological or molecular identification.

The release calls analyzed in two of the three species are probably the first published records for any species of *Ptychadena*. Resemblance of release and advertisement call structure suggests a common origin making them potentially useful for species distinction (e.g. di Tada *et al.* 2001).

The distress calls of the three species share several features, e.g. frequency modulation, dominant frequency two to five times greater than fundamental frequency, many harmonics reaching up to at least 21 kHz, calls emitted with the mouth open. These features are similar to those of the distress calls of other frog species (Schneider & Sinsch 2007). The function of these calls in *Ptychadena* has not been studied but it is likely that the calls are emitted to startle mammalian and avian predators.

Genetic variation and species assignment

Comparison of the 16S rRNA sequence of the Rwandan specimens of *P. anchietae* with sequences from other localities showed slightly increasing differences with geographic

distance. Genetic distance was small (0.2–0.4 %) to specimens from Uganda and western Kenya (Kakamega Forest) and moderately great to those from South Africa (2.1 %). Consequently, there is no doubt about the conspecificity of populations referred to as *P. anchietae*.

The sequences of the 16S gene from Rwandan specimens of *Ptychadena nilotica* are identical to almost identical (p-distance 0.0–0.2 %) to sequences from other localities within the watershed of the Nile from Uganda (Semliki NP, Lake Victoria) and even Cairo (Egypt) which is astonishing given the huge geographic distance of more than 3500 km (directly) or even more than 6600 km along the shores of the river. These populations are also genetically identical to populations from Kenya (Mount Kenya, Taita Hills, Nakuru; p-distance 0.0–0.4%) and differ moderately from sequences from southern Tanzania (Kibebe Farm, p-distance 1.9–2.6%).

The sequences of specimens of *P. porosissima* from Rwanda differ moderately to considerably from those of specimens from Mumba, Tanzania (1.7%) and from South Africa (2.7%), but sequences from a topotypic site ("Angola"; Steindachner 1867) are unavailable. A specimen labelled "*P.* aff. *porosissima* A" from Tanzania and a specimen, from which the second sequence of "*P. porosissima*" from Mumba, Tanzania (AY517601) derives, differ from each other by 6.0 % in the uncorrected p-distance. They neither are conspecific nor belong to the same species as the Rwandan population herein assigned to *P. porosissima*. Specimens of *P. porosissima* are sometimes confused with other species, especially *P. uzungwensis* (Loveridge, 1932) (and vice versa). Even the type series of *P. uzungwensis* as well as the type series of *P. chrysogaster* Laurent, 1954 included specimens of *P. porosissima* (Laurent 1954; Dehling, unpubl. information). Therefore, the Tanzanian specimens assigned to *P. porosissima* are probably simply misidentified members of other species rather than indicators of a cryptic diversity within the nominal taxon *P. porosissima*. Additional sampling of genetic, bioacoustic and morphological data is necessary to unravel the relationships between the populations currently referred to as *P. porosissima*. If the Rwandan populations turn out to belong to a distinct lineage, the name *P. loveridgei* Laurent, 1954 would be available.

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IV

Diversity of *Ptychadena* in Rwanda and taxonomic status of *P. chrysogaster* Laurent, 1954 (Amphibia, Anura, Ptychadenidae)

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Abstract

We assess the diversity of Ptychadena species in Rwanda based on re-examination of voucher specimens in museum collections and our own data from recent assessment of the species composition of amphibian communities in Rwanda. We recognize five species which we allocate to the following available names: *P. anchietae*, *P. chrysogaster*, *P. nilotica*, *P. porosissima*, and *P. uzungwensis*. We did not find evidence for the presence of *P. grandisonae* and *P. oxyrhynchus* which have been listed for the country. The five species can be distinguished by quantitative morphometrics (discriminant analysis, success rate: 100 %) and a number of qualitative characters of external morphology. We provide an identification key to the Rwandan species and describe the morphology of each species in detail. The taxonomic status and the phylogenetic position of *Ptychadena chrysogaster* are further assessed based on the partial sequence of the mitochondrial 16S rRNA. The species differs genetically from available homologous sequences from congeners by an uncorrected p distance of at least 4.2 % and appears to be most closely related to specimens assigned to *P. porosissima*, *P. mahnerti*, "*P*. aff. *uzungwensis*" and "*P*. aff. *bibroni*".

Key words: *P. anchietae*, *P. grandisonae*, *P. nilotica*, *P. porosissima*, *P. uzungwensis*, DNA barcoding, systematics

Introduction

Ridged Frogs of the genus *Ptychadena* Boulenger, 1917 are widespread in sub-Saharan Africa where approximately 50 species occur. Species of the genus share a similar general appearance and many are poorly delimited, having been described based on taxonomically doubtful characters. Several species names have been erroneously considered synonyms of others, thus confusing character diagnoses in subsequent accounts; and some taxa were described based on specimens later found to represent more than one species (e.g. Boulenger 1879; Loveridge 1932; Laurent 1954; Guibé and Lamotte 1957; Schmidt & Inger 1959; Lamotte 1967; Poynton 1970; Rödel 2000; Channing 2001; Channing & Howell 2006; Dehling & Sinsch 2013). Therefore, even the local/regional diversity of these frogs is often difficult to assess. Herein, we address the diversity of Ridged Frogs in Rwanda. We have recently shown that three species (*P. anchietae* [Bocage, 1868], *P. nilotica* [Seetzen, 1855], and *P. porosissima* [Steindachner, 1867]) inhabit the wetlands along the upper Nile (Dehling & Sinsch 2013). Further taxa have been reported from Rwanda and it is currently unclear which species actually occur in the country. Nieden (1913) reported *P. nilotica* (referred to as *Rana mascareniensis* Duméril & Bibron, 1841) from several localities in Rwanda. Based on his own collections, Laurent (1954) reported *P. uzungwensis* (Loveridge 1932) and described *P. chrysogaster* Laurent, 1954 and *P. loveridgei* Laurent, 1954 as new species, the latter now being considered a synonym of *P. porosissima* (Schmidt & Inger 1959). Poynton & Broadley (1985), Channing (2001), and Poynton & Channing (2004) stated that *P. grandisonae* Laurent, 1954 occurs in Rwanda. Fischer & Hinkel (1992) listed only "*P. mascareniensis*" [= *P. nilotica*]. Poynton *et al.* (2004) stated that *P. anchietae* was likely to occur in Rwanda but confirmed records were missing. According to Spawls *et al.* (2006), *P. chrysogaster*, *P. mascareniensis*, and *P. uzungwensis* occur in Rwanda but not *P. anchietae* and *P. porosissima*. Branch (2005) included Rwanda in the geographic range of *P. oxyrhynchus* (Smith, 1849). Recently, we collected *P. anchietae* in Rwanda and resurrected the name *P. nilotica* for the populations which occur along the Nile and in Central Kenya and Tanzania and which had been formerly

referred to as *Rana mascareniensis* or *Ptychadena mascareniensis* (Sinsch *et al.* 2012; Dehling & Sinsch 2013).

In order to clarify how many and which species occur in Rwanda, we re-examined the specimens of *Ptychadena* in the herpetological collection of the Royal Museum for Central Africa in Tervuren, Belgium (RMCA), on which almost all previous Rwandan records are based. We herein report the results and compare the findings to our own data from recent assessment of the composition of amphibian communities at numerous locations in Rwanda. We further assess the taxonomic status and the phylogenetic position of Ptychadena chrysogaster based on examination of most of the available voucher material from Rwanda including the type series and on comparison of the partial sequence of the mitochondrial 16S rRNA gene with homologous sequences of its congeners.

Material and methods

Morphological examination

We examined voucher specimens deposited at RMCA. Additional specimens including our recently collected material are deposited in the collection of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK). See Appendix 4 for a complete list of examined specimens.

For the morphological analysis, we took the following 18 measurements to the nearest 0.1 mm using digital calipers, following Dehling & Sinsch (2013): (1) Snout-vent length (SVL); (2) tibiofibula length (TFL, measured with both knee and tibio-tarsal articulation flexed); (3) thigh length (THL, from vent to knee with thigh being held vertically to median body plane and knee flexed); (4) total hindlimb length (LEG, from vent to tip of fourth toe with leg fully extended and being held vertically to median body plane); (5) tarsus + foot length (TarL, from tibio-tarsal articulation to tip of fourth toe); (6) foot length (FOT, from proximal end of inner metatarsal tubercle to tip of fourth toe); (7) forearm + hand length (ARM, distance from elbow to tip of third finger); (8) hand length (HND, distance from proximal end of inner palmar tubercle to tip of third finger); (9) head width (HW, measured at the level of the jaw joint); (10) head length (HL, distance from posterior end of mandible to tip of snout); (11) interorbital distance (IO, shortest distance between upper eyelids); (12) upper eyelid width

(EW); (13) horizontal eye diameter (ED); (14) horizontal tympanum diameter (TD); (15) eye to nostril distance (EN, distance between anterior margin of eye and centre of nostril); (16) nostril to snout distance (NS, distance between centre of nostril and tip of snout); (17) snout length (SL, distance between anterior margin of eye to tip of snout); (18) internarial distance (NN, distance between centres of nostrils). To avoid an interobserver bias, all measurements were taken by JMD. Additionally, we recorded the following qualitative characters: (1) position of external vocal sac aperture in males; (2) number of longitudinal dorsal dermal ridges; (3) texture of ventral skin; (4) extent of nuptial pads in males; (5) number of supernumerary metacarpal tubercles; (6) size and shape of thenar and palmar tubercles; (7) extent of toe webbing; (8) presence of outer metatarsal tubercle; (9) relative size of inner metatarsal tubercle; (10) ventral colouration; (11) presence of light line on dorsal face of tibia; (12) presence of light band on dorsum; (13) presence of dark brown stripe on preaxial side of tarsus; (14) colour of external dorsal fold; (15) colour pattern on postaxial side of femur. Sex of males was determined by presence of secondary sexual characteristics (vocal slits, nuptial pads), that of females by either examination of gonads through dissection or size (female if larger than smallest 10 percent of adult males). The webbing formulae are given as proposed by Myers & Duellman (1982). Terminology for dermal dorsal ridges and orientation of external vocal sac aperture follows Perret (1979).

Statistical Analyses

Descriptive statistics depended on the outcome of the test for normality. Normally distributed data were described by the arithmetic mean and corresponding standard error and/or range, those deviating significantly by median and range. Principal component analyses were run on the morphometric data set including 18 variables and 89 observations each (*P. anchietae*: 15 males, 3 females; *P. chrysogaster*: 13 males, 10 females; *P. nilotica*: 13 males, 10 females; *P. porosissima*: 11 males, 7 females; *P. uzungwensis*: 6 males, 1 female). We compared the scores obtained for the principal components 2 and 3 describing shape to distinguish taxa without a priori assignment to taxa. The morphometric distances were adjusted for SVL by calculating a linear regression of each variable against SVL and storing the residuals as representatives of size-independent shape variables. This transformed data set was used for discriminant analyses with taxa as predefined groups to optimize distinction. To account for sexual dimorphism, discriminant analyses were run separately for

Figure 17. Males of *Ptychadena* from Rwanda in life. (A) *P. anchietae*, (B) *P. chrysogaster* [Foto: E. Fischer], (C) *P. nilotica*, (D) *P. porosissima*.

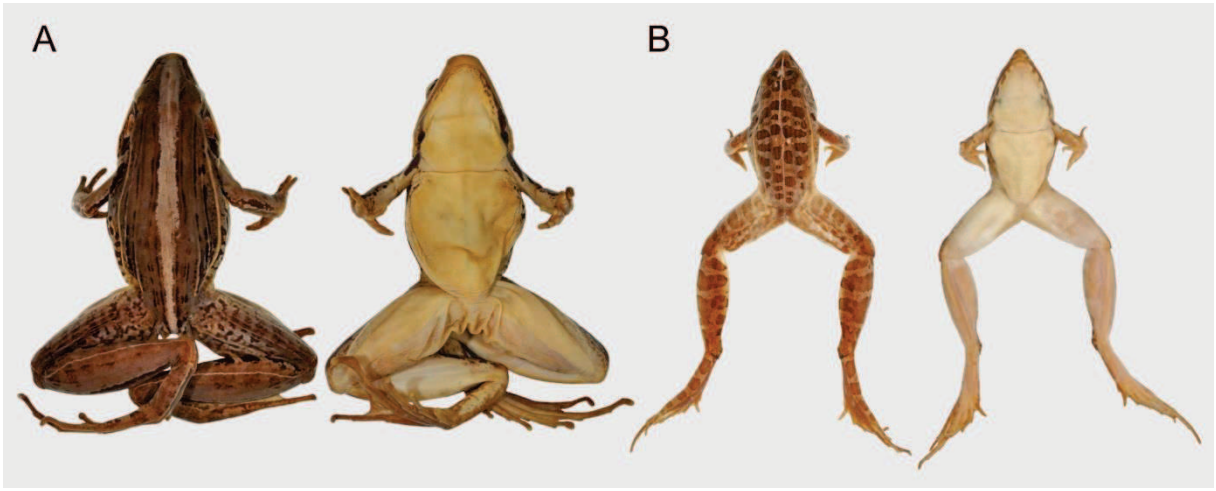

Figure 18. (A) Preserved female holotype of *Ptychadena chrysogaster* (RMCA 109096) from Lac Karago, Rwanda; dorsal view (left) and ventral view (right). (B) Preserved male specimen of *P. uzungwensis* (RMCA 108993-108997) from Munini, Rwanda; dorsal view (left) and ventral view (right). Not to scale.

males (n=58) and females (n=31). Significance level was set at alpha = 0.05. All calculations were based on the procedures of the program package STATGRAPHICS centurion for Windows, version XV.

DNA barcoding and phylogenetic analyses

We isolated DNA from a liver tissue sample from a specimen of *Ptychadena chrysogaster* (ZFMK 58797), collected in southern Rwanda by H. Hinkel in 1993. DNA was used to sequence a fragment of the 16S mitochondrial rRNA gene, a universal marker to barcode amphibian species (Vences *et al.* 2005). Protocols of DNA extraction, PCR, purification, and sequencing follow Dehling & Sinsch (2013). The obtained sequence was compared with those in GenBank using a standard nucleotide-nucleotide BLAST search and with our own sequences from Rwandan specimens and was incorporated into an existing alignment (see Dehling & Sinsch [2013] for a list of sequences and GenBank Accession numbers). Editing and alignment were completed in MEGA5 (Tamura *et al.* 2011). Sequences were trimmed to the same length. The final alignment consisted of 548 base pairs. Calculations of pairwise distances and phylogenetic analysis (Maximum Likelihood) were carried out in MEGA5. Maximum Likelihood analysis was run using the GTR + G + I model and the Nearest-Neighbor-Interchange with 1000 bootstrap replicates.

Results

Examination of specimens suggested that five morphologically distinct species were present in Rwanda to which we assign the following names: *Ptychadena anchietae*, *P. chrysogaster*, *P. nilotica*, *P. porosissima*, and *P. uzungwensis* (Figures 17 & 18). For allocation of specimens to *P. anchietae*, *P. nilotica*, and *P. porosissima* and discussion thereof see Dehling & Sinsch (2013). The examined material included type specimens of both of *P. chrysogaster* and *P. uzungwensis* (Appendix 4). Allocation of other specimens to the latter two species is based on direct comparison with the type material. We reassigned several specimens that had been deposited in the museum collections under wrong names. Noteworthy are two of the paratypes of *P. chrysogaster* (RMCA 41989, 41994) which belong in fact to *P. porosissima*.

Morphological differentiation

The morphometric features of the five species are summarized in Table 11. Principal component analysis yielded three PCs accounting for 89.6% of total variation (Table 12A). PC1 represented variation in size, whereas the shape-related PC2 and PC3 were mainly loaded by features describing head morphology (Table 12B). In females, PC 2 unequivocally

Table 10. Distinguishing qualitative characters of *Ptychadena* species from Rwanda.

Species	*P. anchietae*	*P. chrysogaster*	*P. nilotica*	*P. porosissima*	*P. uzungwensis*
relative length of Toes III and V	tips reaching to knee or slightly beyond, distal subarticular tubercle never reaching knee	tips reaching beyond knee, distal subarticular tubercle reaching knee	tips reaching beyond knee, distal subarticular tubercle reaching knee or beyond	tips reaching to knee or slightly beyond, distal subarticular tubercle never reaching knee	tips reaching to knee or slightly beyond, distal subarticular tubercle never reaching knee
position of vocal sac aperture	inferior, at ventral edge of arm insertion	inferior, at ventral edge of arm insertion	superior, above dorsal edge of arm insertion	inferior, at ventral edge of arm insertion	semi-inferior, at level of centre of arm insertion
spiny tubercles on venter	absent	present in males, very small	absent	present in males, comparatively large	present in males, very small
median dorsal ridge on snout	absent	absent	absent	absent	present
outer metatarsal tubercle	very faintly visible	very faintly visible, rarely distinct	distinctly present, rarely faintly visible	faintly visible, rarely distinct	faintly visible
inner metatarsal tubercle size (Fig. 5)	about half the length of metatarsus of Toe I	less than half the length of metatarsus of Toe I	less than half the length of metatarsus of Toe I	more than half the length of metatarsus of Toe I	about half the length of metatarsus of Toe I
supernumerary metacarpal tubercles (Fig. 5)	one below each finger	one below each finger	only one below Finger IV, often indistinct	one below Fingers I, II, and IV; two, rarely one below Finger III	one below Fingers I and IV, two below Finger II, two to four below Finger III
palmar and thenar tubercles (Fig. 5)	inner and outer palmar tubercle more or less equal in length; thenar tubercle oval, slightly longer than palmar tubercles	outer palmar tubercle longer than inner; thenar tubercle elongate, about as long as outer palmar tubercle	outer palmar tubercle longer than inner; thenar tubercle elongate, about as long as outer palmar tubercle	outer palmar tubercle longer than inner; thenar tubercle elongate, longer than outer palmar tubercle	inner and outer palmar tubercle more or less equal in length; thenar tubercle elongate, longer than palmar tubercles
toe webbing (Fig. 5)	I0.5-2II0.5-2III(0.5-1)-2IV2-0.5V	I2-2.5II(1.5-1.75)-3III(2-2-)(3.25-3+)IV3-(1.5-2)V	I(1.5-1.75)-(2-2.25)II1.5-(2.75-3)III(1.75-2)-3IV2.75-(1-1.5)V	I(1.75-2)-2.25II1.5-3III1.75-(3-3.25)IV3-(1-1.5)V	I2-(2.25-2.5)II1.5-3·III(1.75-2·)-3IV3-(1'-1.25)V
ventral colouration	head white, trunk yellow	head and trunk yellow	head white, mottled with grey; trunk yellow	head and trunk yellow	colours in life unreported
dark brown stripe on preaxial side of tibia	absent	present, continuous or almost continuous	absent in most specimens; few specimens with dark mottling, not forming continuous stripe	absent	absent
light tibial line (Figs. 1& 2)	absent	usually present, rarely absent	present or absent	present	absent
light dorsal band	absent	usually present, rarely absent	present or absent	present or absent	present
dark spots on dorsum (Figs. 1& 2)	usually absent; if present, small and narrow	usually present, small and narrow, sometimes forming longitudinal lines; rarely absent	present, large and wide, sometimes fused with neighboring ones	present, large and wide, sometimes fused with neighboring ones	present, large and wide, often fused with neighboring ones
light, prominent dorsolateral fold (Figs. 1& 2)	usually absent	present	present	present	present
Colour pattern on postaxial side of femur	irregularly delimited, reticulated, longitudinal bands, alternately yellow and dark brown coloured	irregularly delimited, reticulated, longitudinal dark bands on light background; colours in life unreported	relatively sharply delimited longitudinal bands, alternately yellow and black coloured	yellow spots diffusely arranged in longitudinal rows on dark brown background	irregularly delimited, reticulated, longitudinal light bands on dark background; colours in life unreported

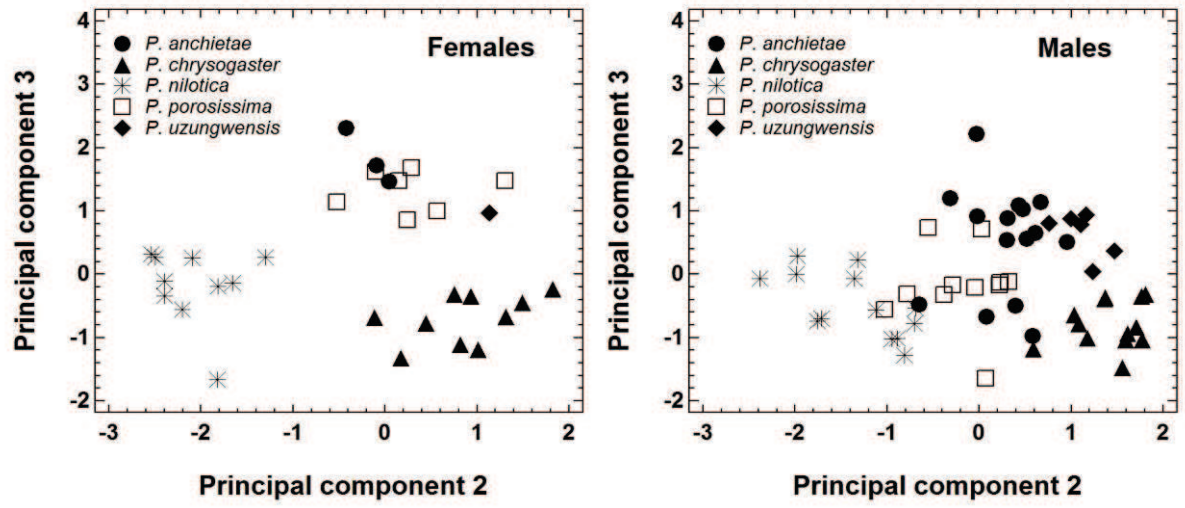

Figure 19. Morphological shape differentiation among 89 specimens representing five *Ptychadena* species, as assessed by principal component analysis (Table 12). (A) Individual scores obtained for 31 females; (B) Individual scores obtained for 58 males.

Table 11. Morphometric features of *Ptychadena* species from Rwanda. Data are given as arithmetic means and minimum and maximum values (in mm).able 11.

Morphometric character	*P. anchietae*		*P. chrysogaster*		*P. nilotica*		*P. porosissima*		*P. uzungwensis*	
	Males N = 15	Females N = 3	Males N = 75/14*	Females N = 23/11*	Males N = 13	Females N = 10	Males N = 11/20*	Females N = 9	Males N = 6	Females N = 1
Snout-vent length	40.4 (38.0–42.4)	49.0 (46.7–51.3)	43.3 (36.3–49.5)	53.7 (48.0–57.7)	42.0 (37.2–45.2)	49.1 (45.6–53.1)	41.2* (37.3–44.5)	46.4 (39.0–52.1)	34.7 (33.3–35.7)	43.3 -
Hindlimb length	79.6 (74.2–84.9)	98.9 (96.0–101.2)	88.1 (83.5–93.0)	108.2 (102.3–114.1)	77.2 (70.1–85.2)	89.6 (78.0–103.9)	78.2* (72.8–85.5)	88.3 (74.3–94.1)	66.7 (61.9–72.7)	81.2 -
Femur length	23.0 (21.9–24.5)	29.0 (28.3–29.6)	23.9 (22.5–25.0)	29.9 (28.1–32.0)	21.5 (19.4–24.0)	25.5 (23.0–28.5)	21.7* (20.2–24.1)	24.4 (19.8–27.6)	18.7 (17.2–19.6)	23.1 -
Tibiofibula length	26.3 (24.4–28.0)	33.1 (31.9–33.8)	28.4 (24.6–32.0)	35.2 (32.4–38.5)	23.4 (21.1–26.1)	27.5 (23.6–32.1)	24.7* (23.3–26.5)	28.7 (25.2–31.0)	21.7 (20.3–23.4)	26.9 -
Tarsus length	34.5 (31.6–36.5)	42.7 (40.6–44.1)	40.9* (38.9–44.1)	49.3* (46.5–51.3)	36.2 (32.4–40.1)	42.8 (35.9–49.6)	35.5 (33.3–38.8)	40.0 (32.6–43.5)	30.5 (27.9–32.2)	36.0 -
Foot length	24.7 (22.4–26.1)	30.5 (29.1–31.3)	28.4 (24.2–30.4)	34.5 (32.9–36.1)	25.7 (22.8–28.2)	29.3 (25.6–33.9)	24.4 (23.0–26.7)	27.4 (23.1–29.3)	21.0 (19.5–21.9)	28.6 -
Forelimb length	16.9 (15.9–18.1)	21.0 (20.0–21.7)	18.3* (17.4–19.1)	22.0* (20.9–23.9)	17.7 (16.0–19.3)	20.5 (17.9–23.7)	17.2 (16.0–18.6)	18.7 (16.1–20.9)	13.5 (12.8–14.4)	16.3 -
Hand length	10.0 (9.5–10.7)	12.3 (12.1–12.6)	10.4* (9.6–11.1)	12.4* (11.8–13.3)	10.4 (9.5–11.6)	11.9 (10.6–13.8)	9.7 (8.9–10.8)	10.7 (9.1–11.7)	7.9 (7.4–8.5)	9.0 -
Head width	13.8 (12.5–15.4)	16.7 (16.4–17.0)	13.9 (12.8–14.7)	16.9 (15.4–17.5)	13.8 (12.4–15.9)	16.4 (14.2–18.8)	14.1 (13.1–15.0)	15.1 (12.5–17.1)	11.4 (11.0–11.7)	13.8 -
Head length	15.5 (14.1–17.6)	18.7 (18.2–19.1)	15.6* (14.7–16.4)	18.6* (17.9–19.8)	16.2 (14.7–18.4)	18.3 (16.6–20.4)	15.5 (14.2–17.8)	17.1 (13.6–19.1)	13.3 (12.3–14.0)	16.1 -
Interorbital distance	2.8 (2.4–3.1)	3.1 (3.0–3.2)	3.5* (3.1–3.9)	4.1* (3.8–4.5)	2.2 (1.9–2.5)	2.5 (2.2 - 2.7)	2.6 (2.3–2.7)	3.1 (2.8–3.6)	2.7 (2.4–3.1)	2.9 -
Eyelid width	2.8 (2.5–3.1)	3.2 (3.0–3.3)	2.7* (2.4–3.0)	3.3* (2.9–3.7)	2.7 (2.3–3.2)	3.0 (2.4–3.5)	2.8 (2.3–3.1)	3.1 (2.6–3.5)	2.6 (2.3–2.8)	2.4 -
Eye diameter	4.3 (3.6–4.9)	5.2 (5.0–5.5)	4.3 (3.7–4.9)	5.0 (4.6–5.6)	4.5 (4.1–5.1)	5.1 (4.8–5.5)	4.2 (3.9–4.5)	4.6 (3.9–5.1)	3.9 (3.6–4.1)	4.5 -
Tympanum diameter	3.3 (2.9–3.6)	4.1 (3.7–4.6)	3.7 (3.2–4.2)	4.5 (4.3–5.1)	3.7 (3.3–4.1)	4.1 (3.7–4.7)	3.1 (2.9–3.49	3.6 (3.3–3.9)	2.9 (2.5–3.3)	3.5 -
Eye–nostril distance	4.1 (3.7–4.3)	5.2 (4.9–5.7)	3.9* (3.5–4.4)	4.6* (4.0–5.0)	3.6 (3.4–4.0)	4.2 (3.7–4.9)	3.5 (3.1–3.9)	4.2 (3.6–4.7)	3.5 (3.2–3.6)	4.2 -
Snout–nostril distance	3.5 (2.9–4.0)	4.4 (4.3–4.5)	3.9* (3.5–4.3)	4.3* (3.9–4.9)	3.4 (2.9–3.8)	3.7 (3.3–4.1)	3.4 (2.9–3.7)	4.1 (2.8–4.7)	3.6 (3.2–3.9)	4.3 -
Internarial distance	3.9 (3.5–4.3)	4.8 (4.7–4.9)	4.3* (4.0–4.5)	4.9* (4.5–5.2)	3.4 (3.0–3.6)	3.7 (2.0–4.5)	3.6 (3.4–4.0)	4.3 (3.7–4.7)	3.0 (2.9–3.2)	3.7 -
Snout length	7.5 (6.6–8.1)	9.5 (9.0–10.1)	7.4* (7.0–7.9)	8.7* (8.4–9.4)	7.0 (6.3–7.7)	7.9 (7.1–9.3)	7.0 (6.3–7.8)	8.1 (6.2–10.1)	6.7 (6.4–7.0)	8.0 -

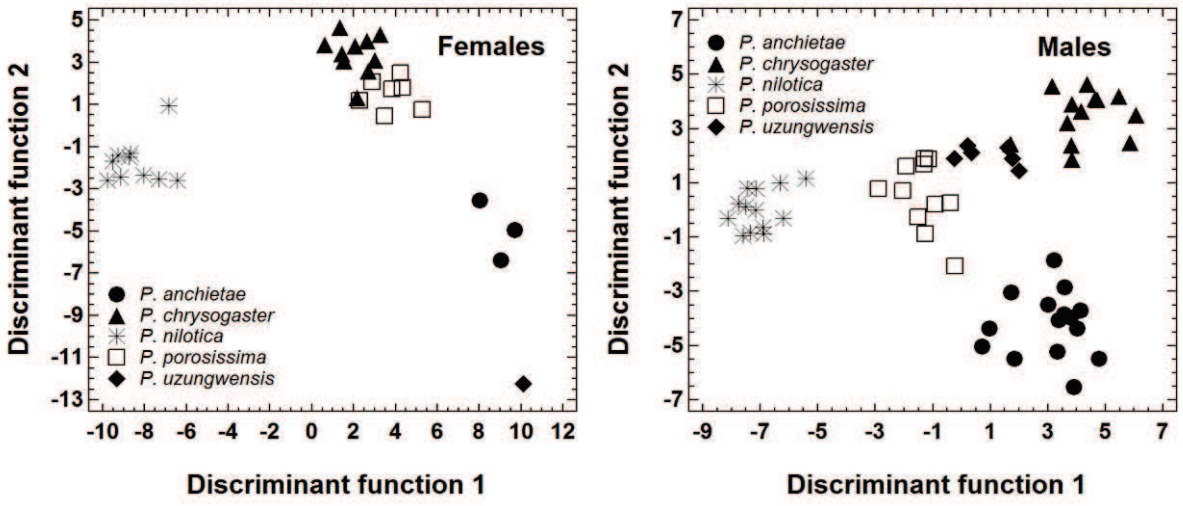

Figure 20. Morphological shape differentiation among 89 specimens representing five *Ptychadena* species, as assessed by discriminant analyses (Statistical details are given in Table 13). (A) Individual scores obtained for 31 females; (B) Individual scores obtained for 58 males.

Table 12. Principal component analysis based on 18 standardized morphometric features of 89 specimens belonging to five *Ptychadena* species from Rwanda. Morphometric parameters accounting strongly for discrimination among species are highlighted in **bold**.

A: Statistical significance			
Principal component	**Eigen-value**	**Relative percentage**	**Cumulative percentage**
1	13.84	76.9	76.9
2	1.46	8.1	85.0
3	0.82	4.6	89.6
B: Standardized coefficients of the principal components			
Parameter	**Principal component 1**	**Principal component 2**	**Principal component 3**
Snout-vent length	0.256	-0.107	-0.084
tibiofibula length	0.253	0.212	-0.011
foot length	0.255	0.046	-0.278
tarsus + foot length	0.257	0.056	-0.246
total hindlimb length	0.261	0.109	-0.137
thigh length	0.260	0.048	-0.033
forearm + hand length	0.253	-0.164	-0.210
hand length	0.244	-0.231	-0.139
head width	0.243	-0.224	-0.016
head length	0.232	**-0.319**	0.105
interorbital distance	0.171	**0.578**	-0.237
upper eyelid width	0.201	-0.142	0.272
horizontal eye diameter	0.211	-0.325	0.162
horizontal tympanum diameter	0.231	-0.112	-0.284
eye to nostril distance	0.228	0.016	**0.353**
nostril to snout distance	0.195	**0.326**	**0.412**
snout length	0.234	0.070	**0.469**
internarial distance	0.227	**0.324**	0.072

Table 13 A. Gender-specific discriminant functions based on 17 SVL-adjusted morphometric features (residuals) to distinguish among five *Ptychadena* species from Rwanda. Statistical significance.

Discriminant function	**Eigen-value**	**Relative percentage**	**Canonical correlation**	**Wilks Lambda**	**Chi-squared**	**Degrees of freedom**	**Statistical significance**
Male 1	19.79	58.76	0.975	0.0003	362.2	68	P < 0.0001
Male 2	8.49	25.23	0.945	0.0079	222.6	48	P < 0.0001
Male 3	3.29	9.77	0.875	0.0750	119.0	30	P < 0.0001
Male 4	2.10	6.24	0.823	0.3223	52.0	14	P < 0.0001
Female 1	45.49	65.38	0.989	0.00005	187.7	68	P < 0.0001
Female 2	14.84	21.33	0.967	0.0023	114.7	48	P < 0.0001
Female 3	6.87	9.89	0.934	0.0377	62.2	30	P < 0.0001
Female 4	2.36	3.40	0.838	0.2973	23.0	14	P = 0.0596

Table 13 B. Gender-specific discriminant functions based on 17 SVL-adjusted morphometric features (residuals) to distinguish among five *Ptychadena* species from Rwanda. Morphometric parameters accounting strongly to discrimination among species are highlighted in **bold**. Standardized coefficients of the discriminant functions.

parameter (residuals)	discriminant function 1 (males)	discriminant function 2 (males)	discriminant function 3 (males)	discriminant function 4 (males)	discriminant function 1 (females)	discriminant function 2 (females)	discriminant function 3 (females)	discriminant function 4 (females)
tibiofibula length	**0.909**	-0.221	0.302	0.871	0.824	-0.143	0.196	0.384
foot length	0.268	-0.253	0.292	**-1.332**	**2.079**	**-3.834**	**-2.308**	**-0.942**
tarsus + foot length	**-0.798**	**2.031**	-0.026	**1.308**	**-3.311**	**4.158**	**1.119**	-0.410
total hindlimb length	0.310	-0.029	-0.147	**-0.929**	-0.617	0.188	0.361	**0.999**
thigh length	0.161	**-1.001**	0.367	-0.108	0.750	0.009	-0.268	0.196
forearm + hand length	-0.172	0.142	**-1.406**	-0.005	0.468	-0.492	0.483	0.105
hand length	**-0.669**	-0.506	0.383	-0.189	-0.879	-0.007	0.088	0.328
head width	0.009	-0.137	-0.370	0.326	-0.778	**0.997**	0.472	0.534
head length	-0.282	-0.341	0.252	-0.389	-0.293	**-1.250**	-0.563	-0.734
interorbital distance	0.366	0.144	0.285	0.136	**1.030**	0.561	-0.269	-0.019
upper eyelid width	-0.022	0.315	-0.221	0.328	**1.579**	0.473	**0.810**	-0.234
horizontal eye diameter	-0.296	-0.048	0.420	-0.193	**1.013**	0.232	**0.808**	0.089
eye to nostril distance	0.417	-0.532	0.473	-0.287	**1.016**	-0.555	0.242	-0.166
nostril to snout distance	0.072	0.529	0.225	0.124	1.162	-0.599	0.727	-0.818
snout length	-0.291	-0.063	-0.123	0.422	-0.176	0.035	-0.337	0.767
internarial distance	**0.666**	0.135	**-0.861**	-0.567	0.594	0.087	-0.026	-0.213
horizontal tympanum diameter	-0.052	-0.157	**0.707**	-0.187	**-1.365**	0.088	-0.621	0.488
Constant	0.909	-0.222	0.302	0.871	0.824	-0.143	0.196	0.384

Table 13 C. Gender-specific discriminant functions based on 17 SVL-adjusted morphometric features (residuals) to distinguish among five *Ptychadena* species from Rwanda. Classification success.

predicted species actual species	*P. anchietae*	*P. chrysogaster*	*P. nilotica*	*P. porosissima*	*P. uzungwensis*
P. anchietae. male female	15 (100%) 3 (100%)	0	0	0	0
P. chrysogaster. male female	0	13 (100%) 10 (100%)	0	0	0
P. nilotica. male female	0	0	13 (100%) 10 (100%)	0	0
P. porosissima. male female	0	0	0	11 (100%) 7 (100%)	0
P. uzungwensis. male female	0	0	0	0	6 (100%) 1 (100%)

distinguished *P. nilotica* from the other taxa, and PC 3 unequivocally distinguished *P. chrysogaster* from *P. anchietae*, *P. porosissima*, and *P. uzungwensis* (Figure 19A). Also, *P. anchietae* and *P. uzungwensis* could be distinguished from each other. However, both species were represented by only few individuals (three and one, respectively) in the analysis. The two species did not differ significantly in shape from *P. porosissima* (Figure

19A). A similar pattern was observed in the analysis of the males (Figure 19B) but males of all five species were generally more similar to each other in shape. Males of *P. nilotica* could be distinguished unequivocally from males of *P. chrysogaster* and *P. uzungwensis* but not from some of the males of *P. anchietae* and *P. porosissima* (Figure 19B). Males of *P. chrysogaster* could be distinguished from males of all other species but some of the males of *P. anchietae* and *P. uzungwensis* were very similar in shape (Figure 19B). Males of *P. anchietae* did not differ significantly in shape from males of *P. nilotica*, *P. porosissima*, and *P. uzungwensis*. Gender-specific discriminant analyses based on the residuals of 17 SVL-adjusted morphometric variables had a classification success of 100 % among the five species in both males and females (Table 13A, B, C; Figure 20A, B).

The five Rwandan species can be distinguished unequivocally from each other using a combination of qualitative morphological characters (Table 10, Figure 21; see also Dehling & Sinsch [2013]). An identification key based on these characters is given below. Detailed morphological descriptions of the species are in Appendix 5.

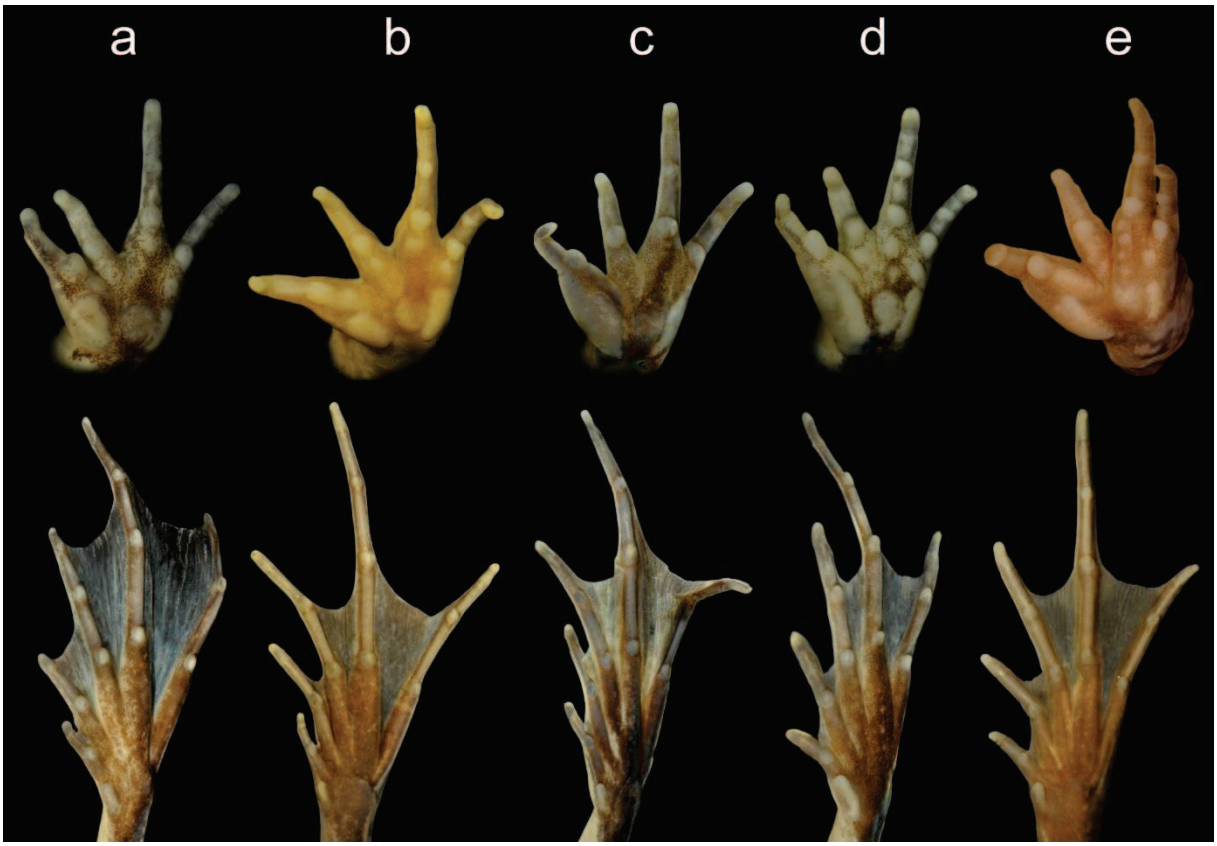

Figure 21. Volar view of hands (top) and plantar view of feet (bottom) of males of *Ptychadena anchietae* (a), *P. chrysogaster* (b), *P. nilotica* (c), *P. porosissima* (d), and *P. uzungwensis* (e) from Rwanda. See also Table 10.

Key to the Rwandan species of *Ptychadena*

1 external vocal sac apertures and nuptial pads on dorsal side of metacarpals and phalanges of Fingers I–III present..... **adult males**...**2**

– external vocal sac apertures and nuptial pads absent.....**adult females and subadults**...**6**

2 vocal sac aperture superior; only one supernumerary metacarpal tubercle proximal to Finger IV, often indistinct; longitudinal, alternately black and yellow coloured bands on postaxial side of femur.....***P. nilotica***
(spiny tubercles on venter absent; inner metatarsal tubercle less than half the length of metatarsus of Toe I; distal subarticular tubercles of Toes III and V reaching to knee; toe webbing I(1.5–1.75)-(2–2.25)II1.5-(2.75–3)III(1.75–2)-3IV2.75-(1–1.5)V; ventral side of head white, mottled with grey)

– vocal sac aperture inferior or semi-inferior; at least one supernumerary metacarpal tubercle proximal to each finger; colouration on postaxial side of femur different.....**3**

3 spiny tubercles on venter absent; toe webbing reaching distal phalanx on postaxial sides of Toes I, II, and III and on preaxial side of Toe V; external dorsal ridge usually not light and prominent..... ***P. anchietae***
(vocal sac aperture inferior; distal subarticular tubercles of Toes III and V never reaching knee; inner metatarsal tubercle about half the length of metatarsus of Toe I; ventral side of head white, trunk yellow; light tibial line and light dorsal band absent; dark spots on dorsum usually absent, if present, small and narrow; irregularly delimited, reticulated, longitudinal, alternately yellow and dark brown coloured bands on postaxial side of femur)

– spiny tubercles on venter present; toe webbing not reaching distal phalanges on toes; external dorsal ridge light and prominent.....**4**

4 median dorsal ridge extending to level between nostrils on dorsal side of snout; vocal sac aperture semi-inferior; two supernumerary metacarpal tubercles proximal to Finger II; inner and outer palmar tubercle more or less equal in length; inner metatarsal tubercle about half the length of metatarsus of Toe I..... ***P. uzungwensis***

– median dorsal ridge extending to level between eyelids only; vocal sac aperture inferior; one supernumerary metacarpal tubercle proximal to Finger II; outer palmar tubercle longer than inner; inner metatarsal tubercle either more than or less than half the length of metatarsus of Toe I **5**

5 foot large, tips of Toes III and V reaching distinctly beyond knee, their distal subarticular

tubercles reaching knee; ventral tubercles tiny, hardly visible with naked eye; inner metatarsal tubercle less than half the length of metatarsus of Toe I; dark brown stripe present on preaxial side of tibia; thenar tubercle approximately as long as outer palmar tubercle; webbing not reaching beyond distal subarticular tubercle on postaxial side of Toe III; dorsal spots small and narrow; irregularly delimited, reticulated, longitudinal dark bands on light background on postaxial side of femur..... ***P. chrysogaster***

– foot smaller, tips of Toes III and V at most reaching slightly beyond knee, their distal subarticular tubercles not reaching knee; ventral tubercles large, visible with naked eye, palpable with finger; inner metatarsal tubercle more than half the length of metatarsus of Toe I; dark brown stripe absent on preaxial side of tibia; thenar tubercle longer than outer palmar tubercle; webbing reaching beyond distal subarticular tubercle on postaxial side of Toe III; dorsal spots large and wide; yellow spots, diffusely arranged in longitudinal rows on dark brown background on postaxial side of femur..... ***P. porosissima***

6 median dorsal ridge extending to level between nostrils on dorsal side of snout; two supernumerary metacarpal tubercles proximal to Finger II.....***P. uzungwensis***
(inner metatarsal tubercle about half the length of metatarsus of Toe I; distal subarticular tubercles of Toes III and V not reaching to knee; toe webbing I2-(2.25–2.5)II1.5-3-III(1.75–2)-3IV3-(1+–1.25)V; light tibial line absent; light dorsal band present; dark spots on dorsum large and wide, often fused with neighboring ones; light, prominent dorsolateral fold present)

– median dorsal ridge extending to level between eyelids only; one or no supernumerary metacarpal tubercle proximal to Finger II.....**7**

7 toe webbing reaching to distal phalanx on postaxial sides of Toes I, II, and III and on preaxial side of Toe V; light prominent external dorsal ridge usually absent; inner metatarsal tubercle about half the length of metatarsus of Toe I..... ***P. anchietae***
(tips of Toes III and V at most reaching slightly beyond knee, their distal subarticular tubercles not reaching knee; ventral side of head white, trunk yellow; light tibial line and light dorsal band absent; dark spots on dorsum usually absent, if present, small and narrow; irregularly delimited, reticulated, longitudinal, alternately yellow and dark brown coloured bands on postaxial side of femur)

– toe webbing not reaching to distal phalanx on toes; light prominent external dorsal ridge present; inner metatarsal tubercle either less than or more than half the length of metatarsus of Toe I **8**

8 inner metatarsal tubercle more than half the length of metatarsus of Toe I; tips of Toes III and V at most reaching slightly beyond knee, their distal subarticular tubercles not reaching knee;

thenar tubercle longer than outer palmar tubercle; yellow spots, diffusely arranged in longitudinal rows on dark brown background on postaxial side of femur .....***P. porosissima***

– inner metatarsal tubercle less than half the length of metatarsus of Toe I; tips of Toes III and V reaching distinctly beyond knee, their distal subarticular tubercles reaching knee; thenar tubercle about as long as outer palmar tubercle; colouration on postaxial side of femur not consisting of spots.....**9**

9 dorsal spots small and narrow; one supernumerary metacarpal tubercle proximal to each finger; ventral side of head and chest yellow; dark brown stripe present on preaxial side of tibia; irregularly delimited, reticulated, longitudinal dark bands on light background on postaxial side of femur; webbing not reaching beyond subarticular tubercle on Toe I ..... ***P. chrysogaster***

– dorsal spots large and wide; only one supernumerary metacarpal tubercle proximal to Finger IV, often indistinct; ventral side of head and chest white; dark brown stripe on preaxial side of tibia absent, few specimens with dark mottling, not forming continuous stripe; longitudinal, alternately black and yellow coloured bands on postaxial side of femur; webbing reaching beyond subarticular tubercle on Toe I..... ***P. nilotica***

Phylogenetic analyses

Comparison of the mitochondrial 16S rRNA gene sequences corroborated the status of *Ptychadena chrysogaster* as a distinct species. The partial sequence of this species differed from all available comparative sequences by an uncorrected p distance of at least 4.2 %. The p distance to sequences from Rwandan specimens of *P. anchietae* and *P. nilotica* was 13.1–13.3 % and 13.6 %, respectively. The lowest values were observed in comparison with specimens assigned to "*P.* aff. *uzungwensis*" (4.2 %), "*P. porosissima*" from South Africa and Rwanda (4.7–4.9 %), "*P.* aff. *porosissima*" from Tanzania (6.0–6.9 %) , "*P. mahnerti*" (6.2 %), and "*P*. aff. *bibroni*" from Gabon (6.9 %). The consensus tree yielded by Maximum-Likelihood analysis indicated that *P. chrysogaster* is most closely related to the aforementioned species (Figure 22). The clade consisting of these species is well supported by bootstrapping (value 0.90; Hillis & Bull 1993), whereas the relationships within the clade are not resolved (bootstrap values <50 %).

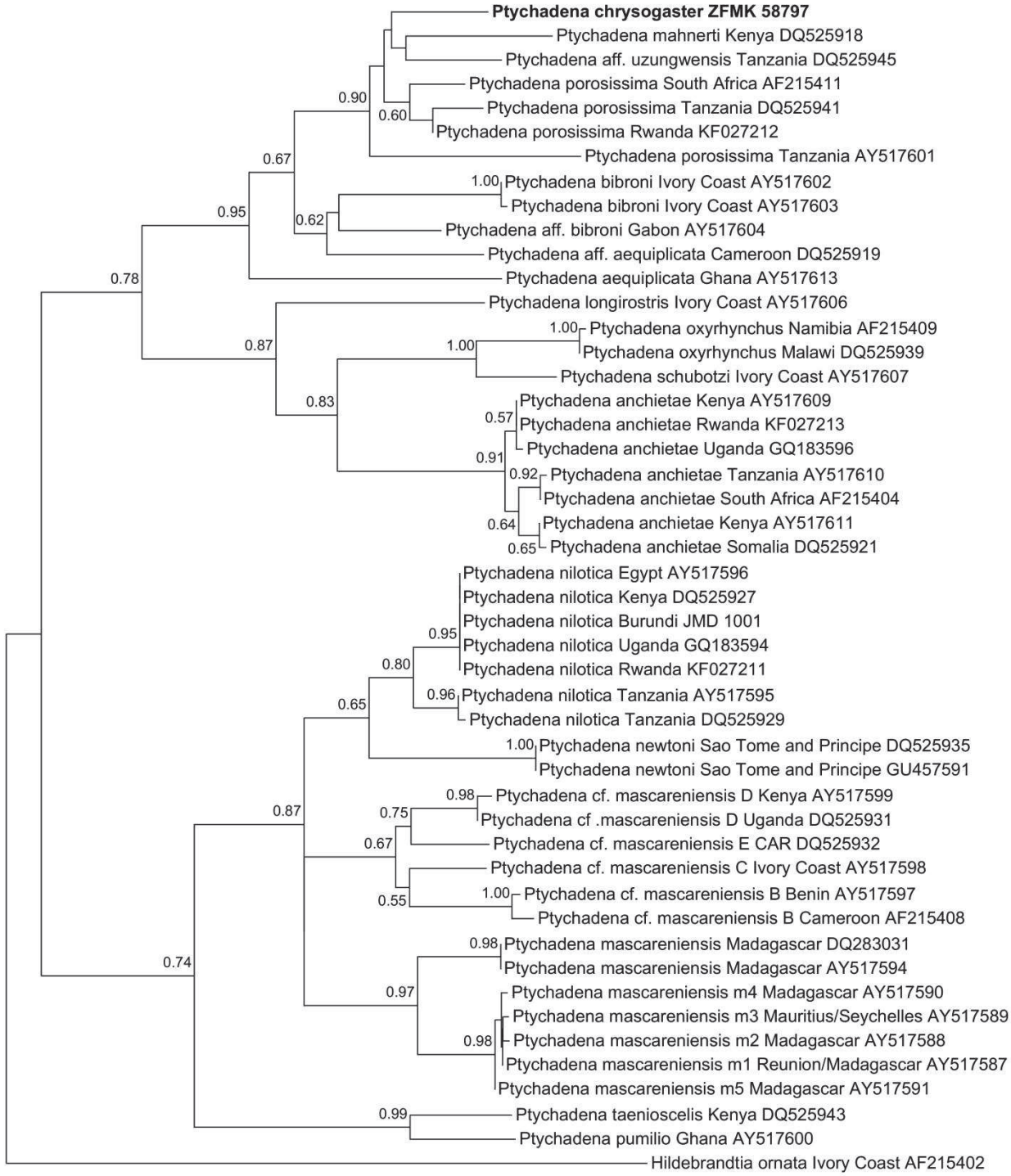

Figure 22. Maximum likelihood phylogram of species in the genus *Ptychadena* and *Hildebrandtia ornata* as outgroup, based on comparison of 548 base pairs of the mitochondrial 16S rRNA gene. Included are specimens from Rwanda and samples taken from GenBank (see Dehling and Sinsch [2013] for a complete list of sequences and accession numbers). Numbers above nodes are bootstrap support values (only values >0.50 are shown).

Discussion

Among the examined material we identified five distinct species of *Ptychadena*: *P. anchietae*, *P. chrysogaster*, *P. nilotica*, *P. porosissima*, and *P. uzungwensis*. The five species are distinguishable from each other unambiguously using quantitative morphometric as well as qualitative morphological characters. The comparison of the partial 16S rRNA sequence of a specimen of *P. chrysogaster* with sequences from congeners corroborated its distinct specific status. Sequences from specimens of *P. anchietae*, *P. nilotica*, and *P. porosissima* from Rwanda differ from each other considerably by an uncorrected p distance of more than 10 % (Sinsch *et al.* 2012; Dehling & Sinsch 2013). Unfortunately, no homologous sequence of *P. uzungwensis* from Rwanda and only a sequence of a specimen with doubtful identity from Tanzania (*P.* aff. *uzungwensis*, GenBank #DQ525945) were available for comparison. We did not find any *Ptychadena* individual collected in Rwanda which was assignable to *P. oxyrhynchus* in the collections of the RMCA and the ZFMK. Three specimens from Kisenyi (= Gisenyi, nowadays Rubavu; RMCA 51565–67), Rwanda, had been deposited under the name *P. oxyrhynchus* but were re-identified as males of *P. anchietae*. Nieden (1913) reported on several specimens of *P. oxyrhynchus* (as *Rana oxyrhyncha*) which Schubotz had collected in what today is northwestern Tanzania, at Kifumbiro and in the Mpororo area, close to the present border with Rwanda. We have not examined Schubotz' material but if his specimens are indeed *P. oxyrhynchus* it is possible that the species can be found in Rwanda as well, given its vast distribution in eastern Africa and the fact that the herpetofauna of the northeastern part of Rwanda has been poorly sampled so far.

There is no specimen of *P. grandisonae* among the material Laurent collected in Rwanda. Laurent (1954) described the species based on type specimens from Muita in Angola, from Kanzenze and Kansenia in Katanga (DRC), and from Bitare in "Urundi" [= Burundi]. The latter is a town in central Burundi, about 20 km north of Gitega at 3°15'S, 29°54'E. Several authors, however, have stated that *P. grandisonae* occurs in Rwanda (Poynton & Broadley 1985; Channing 2001; Poynton & Channing 2004), and Poynton & Channing (2004) even stated that there is no record from Burundi. This misinformation was very likely caused by Laurent himself in a paper which was cited by all above mentioned authors, an account on the "Reptiles et Amphibiens de l'Angola" (Laurent 1964). Therein, Laurent (1964: 139) cited one of the type localities of *P. grandisonae* wrongly as "Bitare (Ruanda)". On the same page, the

locality is correctly given as “Bitare […] (Urundi)” in the account on *Ptychadena uzungwensis*. Thus, the often cited record of *P. grandisonae* from Rwanda in fact refers to specimens from Burundi (RMCA 109036–37; Appendix 4). So far, there is no evidence for the occurrence of *P. grandisonae* in Rwanda.

The available evidence indicates that only five species of *Ptychadena* occur in Rwanda. At present, three of these species (*P. anchietae*, *P. nilotica*, and *P. porosissima*) are widespread and can be found abundantly in both wetlands of the eastern lowland between 1300 and 2000 m elevation which drains into the Nile River and the western lowland on the shore of Lake Kivu which drains into the Congo River. The species inhabit higher elevations of up to 2300 m in deforested, cultivated areas, but are absent from dense forest habitats at similar elevations which at present only remain in the Volcano and Nyungwe National Parks and in the Gishwati Forest. *P. uzungwensis* is known from Rwanda from only few specimens, five males from “Kumunini” [= Munini, South Province, 2°42'S, 29°32'E] and a female from “Astrida” [= Butare/Huye, South Province, 2°36'S, 29°44'E], collected in 1952 and 1951, respectively, and has not been found since. Assuming the species is still extant in Rwanda, its distribution is apparently restricted to the south of the country.

There are large series of *P. chrysogaster* from various localities in Rwanda in the collection of the RMCA (Appendix 4), collected by Laurent in 1951–1952, indicating that the species was abundant at that time. We repeatedly conducted surveys at several of these localities including the type locality at Lac Karago (1°37'S, 29°30'E) but did not encounter individuals of *P. chrysogaster*. Our survey periods (February to April, September to October) were at similar times of the year to those of Laurent (January, February, and October). Species of *Ptychadena* are among the most conspicuous frogs in areas they inhabit, usually occurring in high numbers and easy to detect. Although the absence of a species from a certain area cannot be proven ultimately, our observations indicate that *P. chrysogaster* has disappeared from these areas or at least is much less common than it used to be. The human population in Rwanda has grown from little more than 2 million people in 1950 to approximately 11 million in 2011 (United Nations, Population Division 2011). Nowadays almost every cultivatable area except the three national parks and few small forest patches has been altered to farmland (pers. observation). Gishwati Forest has been reduced to a small patch of a few square kilometres, but until the mid-1990s it had covered a large area in

northwestern Rwanda and its extensions reached the shores of Lac Karago. The former presence of forest habitat at the lake is still indicated by the occurrence of two forest-dwelling frog species, *Hyperolius castaneus* and *Leptopelis kivuensis*, which call from bushes and groups of small trees at the shore of the river (own unpublished data; see also Sinsch *et al.* 2011). Judging from its collection sites, *P. chrysogaster* appears to occur primarily in wetlands within or at the edge of forest. Instead of *P. chrysogaster*, we found *P. nilotica* at Lac Karago and *P. nilotica* and *P. anchietae* in Huye (formerly Astrida and Butare) and in the vicinity of Muzanze (formerly Ruhengeri) during our recent surveys, two species that Laurent had not collected in Rwanda. Both species are known to be able to cope with habitat alteration and are often found in disturbed habitats and in human settlements (Spawls *et al.* 2006; pers. observation). It is possible that habitat alteration promoted population decline in *P. chrysogaster* and its replacement by other species. The distribution of *P. chrysogaster* in Rwanda is currently under study. If our preliminary observations are affirmed, the Red List classification of *P. chrysogaster* would have to be changed to a "threatened" category and it would call for conservation measures.

Our recent efforts to untangle the diversity of *Ptychadena* in Rwanda are a first step to clarify the complicated taxonomy of the genus in sub-Saharan Africa. The results of our studies show that species of *Ptychadena* can be easily distinguished, if standardized diagnostic schemes are applied, which has also been demonstrated by previous studies (e.g. Perret 1979; Bwong *et al.* 2009). Integrative approaches combining data from morphology, bioacoustics, and molecular genetics will be the best way to address the existing taxonomic problems. Doubtful delimitations of *Ptychadena* species were often caused by assigning specimens to the wrong species based on non-diagnostic characters. Thereby, states of possible diagnostic characters were mixed up in subsequent accounts on these species, rendering them difficult to distinguish from each other. In the case of *P. chrysogaster*, two of its paratypes were in fact *P. porosissima*, a severe confusion by Laurent (1954). The latter species, however, was described by Laurent in the same paper as yet another new species, *P. loveridgei*. When even the describer cannot reliably distinguish the species, subsequent workers must fail. When reviewing the taxonomic status of certain species, it is mandatory to critically question decisions made by earlier authors by carefully re-evaluating proposed diagnostic characters and re-examining not only the holotypes, but also the material on which accounts discussing the variation within species and keys to species were based.

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V

An African glass frog: A new *Hyperolius* species (Anura: Hyperoliidae) from Nyungwe National Park, southern Rwanda

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Abstract

A new species of *Hyperolius* is described from Nyungwe National Park in southern Rwanda. The new species differs from its congeners by a unique combination of morphological characters, including a light green dorsum and a transparent ventral skin resembling glass frogs of the Neotropical family Centrolenidae, blue-coloured bones, reduced toe webbing, relatively wide head, acuminate snout, small to medium size (SUL of adult males 18.8–23.2 mm), and the presence of nuptial pads. The advertisement call differs from all calls of other species of the genus that have been analyzed. Comparison of the mitochondrial 16S rRNA gene showed a relatively close relationship to *H. castaneus*, *H. cystocandicans*, *H. discodactylus*, *H. frontalis*, and *H. lateralis*. The 16S sequence of the new species differs by at least 4.5% in the uncorrected p-distance from all available sequences of other species of the genus.

Incamake

Ubwoko bushya bwa Hyperolius buboneka muri parike nasiynali ya Nyungwe mu majyepfo yu Rwanda. Ubu bwoko bushya butandukaniye na bugenzi bwabwo gusa kurusobe

rw'miterere yabwo, harimo dorsum ifite ibara ry'cyatsi cyerurutse n'ruhu rubonerana rwo kunda, amagufwa afite ibara ry'bururu, n'tunodufatanijwe n'gahu, umutwe wenda kuba munini, umunwa uurungushuye, umubyimba uri hagati ya 18,8 mm kugera kuri 23,3 mm ku ngabo nkuru, ikagira n'magaragamba ku ruhu. Kuzitangaza kwazo byagiye bitandukana n'matangazo yabaye kubundi bwoko bwazo. Ugereanije n'miterere yazo idahinduka (mitochondrial 16S rRNA gene) niyizindi usanga bifitanye isano ya bugufi na *H. castaneus*, *H.cystocandicans*, *H.discodactylus*, *H.frontalis* na *H. lateralis*. Urukurikirane rwa 16S y'bu bwoko bushya rutandukanye kuri 4,5% ugereranije n'nkurikirana zabaye z'bundi bwoko busa n'bu.

Key words: *Hyperolius jackie* sp. n., Albertine Rift, endemism, advertisement call, species barcoding, Amphibia

Introduction

Among the sub-saharan amphibians, reed frogs (*Hyperolius*) have undergone the highest degree of radiation. Currently, about 120 species are considered valid (Frost 2011). Taxonomy of this genus is difficult because species are often poor in external morphological characters that allow differentiation, intraspecific variation of characters can be high, and the original descriptions of a number of species do not provide useful diagnostic characters. Furthermore, the type specimens of several species are considered lost or have been damaged, hindering or complicating their re-examination. The introduction of other techniques, especially the comparison of the advertisement call and the use of molecular genetics has simplified in many cases the differentiation between taxa and has led to the discovery of cryptic diversity within the genus (e.g. Wieczorek *et al.* 2001; Channing *et al.* 2002; Rödel *et al.* 2010; Schick *et al.* 2010).

The Central African mountain ranges of the Albertine Rift are among the biodiversity centres of the African continent and harbour a huge number of locally endemic species (Plumptre *et al.* 2007). One of the largest remaining forests of this area is the Nyungwe Forest in southern Rwanda. Most of the field work on its herpetofauna was conducted at the beginning of the 20th century, the results of which were mainly published by Nieden (1911, 1913) and Ahl (1931a).

During field work in the Nyungwe National Park in southern Rwanda, I collected a small series of a species of reed frog which differs morphologically from all described species of the genus from Central Africa. Moreover, its call characteristics as well as the 16S rRNA sequence distinguish the species from all other species of the genus for which comparative information is available. Therefore, I describe formally the species herein as new to science.

Material and methods

I collected specimens during field work in Rwanda in March and April 2011 and March 2012. Specimens were collected at night and photographed in the habitat prior to collection. Latitude and longitude of the type locality were determined using a Garmin GPSmap 60CSx.

The specimens were fixed and stored in 70% ethanol. I took the following measurements (to the nearest 0.1 mm) with digital calipers under a dissecting microscope: snout-urostyle length (SUL, from tip of snout to vent); tibiofibula length (TFL, measured with both knee and tibio-tarsal articulation flexed); total leg length (LEG, from vent to tip of fourth toe with leg fully extended at right angle to body axis); tarsus + foot length (TarL, from tibiotarsal articulation to tip of fourth toe); thigh length (THL, from vent to knee with thigh being held laterally at right angle to the body and knee flexed); foot length (FOT, from proximal end of inner metatarsal tubercle to tip of fourth toe); hand length (HND, from proximal end of thenar tubercle to tip of third finger); head width (HW, measured at corners of the mouth); head length (HL, from posterior end of mandible to tip of snout); horizontal eye diameter (ED); horizontal tympanum diameter (TD); eye-tympanum distance (ET, shortest distance between tympanum and orbit); eye-to-nostril distance (EN, from anterior edge of orbit to centre of nostril); nostril-snout distance (NS, from centre of nostril to tip of snout); internarial distance (NN, distance between centres of nostril); snout length (SL, from anterior edge of orbit to tip of snout); eye distance (EE, distance between anterior edges of eyes); interorbital distance (IO, shortest distance between upper eyelids); upper eyelid width (EW, maximal width of upper eyelid), gular flap width (GF, maximum width of gular flap). The webbing formulae are given as proposed by Myers and Duellman (1982).

Liver tissue samples were taken from the specimens and stored separately in 98% ethanol. They were used to sequence a fragment of the 16S mitochondrial rRNA gene, a suggested

universal marker to barcode amphibians for species allocation (Vences *et al.* 2005). DNA was extracted 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 16S mitochondrial rRNA using standard primers (Palumbi *et al.* 2002) and standard PCR conditions (Palumbi 1996) with the following thermal cycle profile: 120 s at 94 °C, followed by 33 cycles of 94 °C for 30 s, 53 °C 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 Highpure PCR Product Purification Kit (Roche Diagnostics). Sequencing was performed with the DYEnamic ET Terminator Cycle Sequencing Premixkit (GE Healthcare, Munich, Germany) for sequencing reactions run on a MegaBACE 1000 automated sequencer (GE Healthcare). DNA sequences were corrected and aligned by eye. The obtained sequences were compared with those in GenBank using a standard nucleotide-nucleotide BLAST search. Editing and analyses of pairwise distances were completed in MEGA5 (Tamura *et al.* 2011). Sequences were trimmed to the same length. The final alignment consisted of 487 base pairs.

Advertisement calls were recorded with a Sony PCM–D50 Linear PCM Recorder with stereo microphones, Sony Deutschland GmbH, Cologne. Stereo recordings were converted to mono at a sampling rate of 44.1 kHz and resolution of 16 bits. Sonagrams and frequency analyses were obtained using ADOBE Audition 1.5 and applying Blackman-Harris Fast Fourier transformation with a FFT size of 1024 Hz. Temporal data were obtained from the oscillograms and frequency information was obtained from the audiospectrograms. Call properties used for comparison were taken from my own unpublished data from Rwandan specimens and call descriptions of Schiøtz (1999), Köhler *et al.* (2005a), and Channing & Howell (2006).

For comparison of morphological characters I examined vouchers of all Hyperolius species from Rwanda and similar species from adjacent areas including the types of species considered synonyms (see Appendix 6). These vouchers are deposited in the collections of the Royal Museum for Central Africa, Tervuren, Belgium (Musée royal de l'Afrique centrale; MRAC), the Museum für Naturkunde, Berlin, Germany (ZMB) and in my working collection (JMD). Further museum abbreviations used in the text are: California Academy of Sciences, San Francisco, USA (CAS), Zoologisches Forschungsmuseum Alexander Koenig, Bonn,

Germany (ZFMK). Additional information of characters states of compared species were taken from Laurent (1950a, 1951, 1972a), Richards and Schiøtz (1977), Schiøtz (1999), and Channing and Howell (2006).

Systematics

***Hyperolius jackie* sp. n.**

Jackie's Reed Frog (Figures 23 & 24)

Holotype. ZMB 77476 (ex JMD 674), adult male, from a natural pond at Karamba (S 2°28'44.28'', E 29°06'44.50'', 1940 m a.s.l.), Nyungwe National Park, Rwanda, collected on 20 March 2011 at 19:25 h by J. M. Dehling.

Paratopotypes. ZMB 77477–77480 (ex JMD 671– 673, 675), four adult males, collected on 19 & 20 March 2011; ZMB 77481 (ex JMD 726), adult male, collected on 3 April 2011; ZMB 77782 (ex JMD 799), adult male, collected on 18 March 2012; ZMB 77783 (ex JMD 800), adult male, collected on 24 March 2012; otherwise same collection details as holotype.

Figure 23. Holotype of *Hyperolius jackie* sp. n. in life (ZMB 77476). Photograph taken in situ.

Diagnosis. The new species is assigned to *Hyperolius* for showing the following characters considered diagnostic for the genus (Schiøtz 1999, Channing & Howell 2006): Pupil horizontal, skin smooth, tips of fingers and toes expanded, males with disc-shaped gular flap. *Hyperolius jackie* is distinguishable from its congeners by the combination of the following characters: (1) size small to medium, SUL of adult males 18.8–23.2 mm, (2) head relatively wide, (3) snout acuminate in dorsal view, rounded in profile, (4) canthus rostralis weakly distinct, (5) tympanum externally discernible, (6) dorsal surfaces smooth with few tiny tubercles, (7) finger and toe webbing not extensive, formulae being **I**2.25-(2.25–2.75)**II**(2^+–2.25)-(3–3^+)**III**2.5-(2^+–2.25)**IV** and **I**(2–2^+)-(2^+–2.5)**II**1.5-3**III**(2–2^-)-(3–3^+)**IV**(3–3^-)-(1.75–2^-)**V**, respectively, (8) nuptial pads present in males, (9) pectoral and brachial glands absent in males, (10) hind limbs moderately long, (11) dorsum light green in life with very small brown dots, (12) ventral skin transparent, (13) bones blue, visible through skin, (14) advertisement call consisting of a single pulse group, repeated in series of 2–13 calls, pulse group consisting of 10–18 pulses and lasting 54–107 ms, dominant frequency at 2650– 3150 Hz, (15) 16S rRNA sequence differing by at least 4.5 % in the uncorrected p distance from all currently available sequences of other species of the genus.

Description of holotype (Figures 23, 24). Measurements are provided in Table 14. Body moderately sturdy, widest at temporal region, tapering to groin; head large (HL/SUL 0.35, HW/SUL 0.36), slightly wider than long (HW/HL 1.03); snout long (SL/HL 0.48), acuminate in dorsal view, rounded in profile, slightly projecting beyond lower jaw, wider than long (SL/EE 0.80); canthus rostralis weakly distinct between eye and nostril, straight-lined; loreal region oblique, slightly concave; nostrils rounded, directed anterolaterally; situated closer to tip of snout than to eye (EN/NS 1.34), separated from each other by distance subequal to distance between eye and nostril (NN/EN 0.97); eyes directed anterolaterally, protruding, large (ED/HL 0.39), its diameter shorter than snout (ED/SL 0.82); interorbital distance wider than upper eyelid (IO/EW 1.61) and greater than internarial distance (IO/NN 1.25); tympanum concealed under thick layer of skin but discernible, separated from eye by 29% of its diameter; tympanum diameter slightly more than one-third of eye diameter (TD/ED 0.35), upper jaw with dentition; teeth on premaxilla somewhat larger than those on maxilla; choanae small, oval, located far anterolaterally at margins of roof of the mouth, concealed by upper jaw for about the half in ventral view; vomer processes and teeth absent; tongue long (5.6 mm) and broad (4.0 mm at widest point), bilobed for slightly less than one-third of

Table 14. Measurements and proportions of the type series of *Hyperolius jackie* sp. n. For abbreviations see Material & Methods.

ZMB	77476	77477	77478	77479	77480	77481	77782	77783
Status	Holotypp	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype
SUL	23.1	20.1	21.2	23.2	19.0	21.5	18.8	19.8
TFL	12.1	10.2	10.8	11.8	9.7	10.8	9.1	9.9
LEG	38.2	32.3	33.7	37.6	30.8	34.9	28.8	32.0
TarL	16.6	14.1	14.8	16.9	14.0	15.8	13.3	14.1
THL	11.4	10.0	10.1	11.0	9.3	10.5	8.4	9.6
FOT	10.1	8.4	8.8	10.3	8.3	9.9	8.1	8.9
HND	6.5	5.8	6.0	6.9	5.3	6.8	5.7	5.7
HW	8.3	7.5	7.5	8.2	7.0	8.1	7.0	7.4
HL	8.1	7.2	6.9	8.1	6.5	7.4	6.8	6.9
ED	3.2	2.9	2.9	3.1	2.6	2.9	2.8	2.8
TD	1.1	1.1	1.1	1.1	0.9	1.0	0.9	1.0
ET	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
EN	2.3	1.9	1.9	2.3	1.8	2.1	1.7	1.8
NS	1.7	1.6	1.6	1.7	1.5	1.8	1.3	1.5
NN	2.3	2.1	2.2	2.3	2.0	2.3	1.9	2.0
SL	3.8	3.1	3.2	3.8	3.0	3.6	2.9	3.0
EE	4.8	4.2	4.2	5.0	4.1	4.7	4.1	4.1
IO	2.8	2.4	2.5	2.7	2.3	2.6	2.3	2.5
EW	1.7	1.7	1.6	1.8	1.7	1.7	1.7	1.6
GF	6.0	5.7	5.8	6.0	5.2	5.3	5.5	5.4
TFL/SUL	0.52	0.51	0.51	0.51	0.51	0.50	0.48	0.50
FOT/SUL	0.44	0.42	0.42	0.44	0.44	0.46	0.43	0.45
LEG/SUL	1.65	1.61	1.59	1.62	1.62	1.62	1.53	1.61
THL/TFL	0.94	0.98	0.94	0.93	0.96	0.97	0.93	0.97
FOT/TFL	0.84	0.82	0.82	0.88	0.86	0.91	0.89	0.90
HND/SUL	0.28	0.29	0.28	0.30	0.28	0.31	0.30	0.29
HW/SUL	0.36	0.37	0.36	0.36	0.37	0.37	0.37	0.37
HL/SUL	0.35	0.36	0.33	0.35	0.34	0.34	0.36	0.35
HW/HL	1.03	1.03	1.09	1.02	1.06	1.09	1.04	1.08
SL/HL	0.48	0.43	0.46	0.47	0.45	0.48	0.42	0.43
SL/EE	0.80	0.73	0.77	0.77	0.73	0.75	0.70	0.72
IO/NN	1.25	1.12	1.18	1.17	1.17	1.16	1.17	1.25
IO/EW	1.61	1.40	1.55	1.46	1.37	1.53	1.31	1.56
TD/ED	0.35	0.38	0.37	0.37	0.34	0.34	0.32	0.35
EN/NS	1.34	1.16	1.22	1.34	1.22	1.20	1.28	1.18
NN/EN	0.97	1.10	1.14	1.00	1.12	1.07	1.15	1.10
ED/SL	0.82	0.95	0.91	0.80	0.89	0.82	0.97	0.93
ED/HL	0.39	0.40	0.42	0.38	0.40	0.39	0.41	0.40
ED/EN	1.36	1.53	1.54	1.32	1.49	1.37	1.64	1.52
IO/HW	0.34	0.32	0.34	0.33	0.34	0.33	0.32	0.34

its length, free distally for half its length; median lingual process absent; vocal sac single, median, subgular; gular flap medially arranged as subcircular area of thickened, glandular

skin; vocal sac aperture on each side of the mouth, slit-like, long, directed posterolaterally, situated closer to eustachian tube openings than to base of tongue.

Dorsal surfaces of head, trunk and limbs smooth with few scattered tiny low, pointed tubercles; ventral side of limbs and gular region smooth, chin and abdomen slightly more areolate; supratympanic fold absent.

Forelimbs slender; hand moderately large (HND/SUL 0.28); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I < II < IV < III; subarticular tubercles rounded, well developed, numbering one on Fingers I and II, two on Fingers III and IV, with proximal tubercles on Fingers III and IV much smaller than distal ones; webbing formula of the hand **I**2.25-2.5**II**2^{+}-3^{+}**III**2.5-2.25**IV**; thenar tubercle distinct, about one-sixth the size of base of thumb; palmar tubercles absent; nuptial pad as faintly discernible callosity with few loosely scattered, tiny pointed tubercles on preaxial half of the dorsal surface of Finger I.

Hind limbs slender, moderately long (LEG/SUL 1.65); tibio-tarsal articulation reaching to level of nostrils when legs are adpressed forwardly to body; tibiofibula moderately long (TFL/SUL 0.52), longer than thigh (TFL/THL 1.06); heels overlapping each other slightly with knees flexed and thighs held at right angle to median plane; foot shorter than tibiofibula (FOT/TFL 0.84); relative length of toes: I < II < III < V < IV; discs of toes smaller than those of fingers; subarticular tubercles numbering one on Toes I and II, two on Toes III and V, and three on Toe IV; pedal webbing formula **I**2-2.5**II**1.5-3**III**2-3**IV**3-1.75**V**; inner metatarsal tubercle small (0.9 mm), 27% length of Toe I (3.3 mm), oval, little prominent; outer one almost circular, low and less distinct, covering larger area than inner one.

Colouration in life. Dorsal side of head, trunk, forelimbs, tibiofibula and tarsus light green with very small brown dots; thigh unpigmented with a yellowish-green dye; ventral side superficially unpigmented, translucent; chin appearing green; gular region and anterior part of chest including lateral sides around insertion of forelimbs, groin, and areas below pelvis, knee, and tibio-tarsal articulation greenish blue; posterior part of chest and abdomen yellow, ventral side of limbs yellowish-green; dorsal side of toes, finger tips and toe tips, and toe webbing greenish yellow; dots on dorsum brown to coppery, loosely scattered on trunk and limbs, most densely around nostril, along canthus rostralis and on upper eyelid; few spots on trunk and tibia slightly enlarged; whitish mottling along edges of both upper and

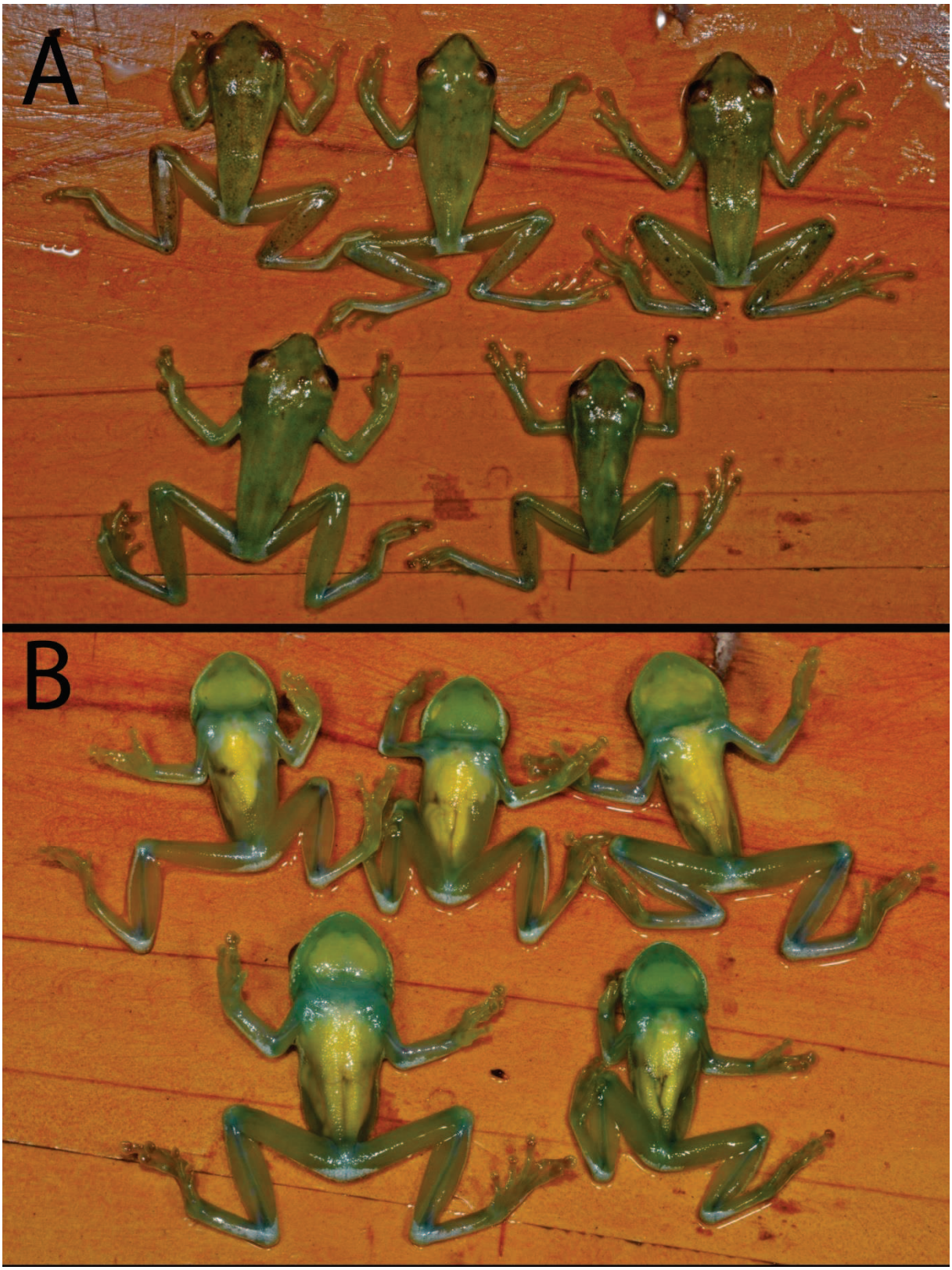

Figure 24. Dorsal view (A) and ventral view (B) of five type specimens of *Hyperolius jackie* sp. n. Top row from left to right: ZMB 77477–77479 (paratypes), bottom row from left to right: ZMB 77476 (holotype), 77480 (paratype).

Table 15. Uncorrected p-distance between *Hyperolius jackie* sp. n. (paratype ZMB 77481, GenBank accession number JQ966571) and other species of *Hyperolius* from Rwanda and adjacent areas, based on 487 base pairs of mitochondrial 16S ribosomal RNA.

Species	Origin	GenBank #	Voucher	p-distance [%]
Hyperolius jackie (paratype)	Nyungwe National Park, Rwanda	----------	ZMB 77480	0.4
Hyperolius castaneus	Nyungwe National Park, Rwanda	JQ423936	ZMB 77537	4.5
Hyperolius cf. *cinnamomeoventris*	Butare, Rwanda	JQ966568	ZMB 77533	9.6
Hyperolius cystocandicans	Mt. Kenya, Kenya	FJ594079	ZFMK 77611	6.6
Hyperolius discodactylus	Nyungwe National Park, Rwanda	JQ966565	ZMB 77536	6.4
Hyperolius alticola (considered a synonym of *H. discodactylus*)	Bwindi Impenetrable National Park, Uganda	DQ283225	CAS 202047	7.1
Hyperolius frontalis	data not provided	AY603986	CAS 201986	5.8
Hyperolius kivuensis	Butare, Rwanda	JQ966567	ZMB 77532	10.3
Hyperolius lateralis	Butare, Rwanda	JQ966569	ZMB 77534	7.5
Hyperolius sp. (*viridiflavus-group*)	Gitarama, Rwanda	JQ966570	ZMB 77535	14.5
Hyperolius sp. (*viridiflavus-group*)	Mizingo, Rwanda	JQ966566	ZMB 77531	14.8
Hyperolius sp. (*nasutus*-group)	Butare, Rwanda	----------	ZMB 77141	16.8

jaw, along the border of pigmented dorsal side with unpigmented ventral side, on postaxial sides of forelimb and tarsus and preaxial side of tibiofibula, in infrananal region, and at tibio-tarsal articulation; iris coppery; larger bones (femur, tibiofibula, tarsus, humerus, radioulna, bones of hand and foot) blue, visible through skin (Figure 24).

Colouration in preservative. All colours have faded to white except dark dorsal dots and spots. Larger bones still have a bluish dye.

Variation. Two of the paratypes (ZMB 77477, 77479) had a yellowish-green dorsum at night. Two of the smaller paratypes (ZMB 77477, 77480) have a narrow dorsolateral band running from the posterior edge of the upper eyelid to about halfway between the insertion of arm and the insertion of leg (Figure 24). A dorsolateral band is faintly visible in the smallest (ZMB 77782) and a somewhat larger paratype (ZMB 77478) and lacking in a medium-sized (ZMB 77783) and the two largest paratypes (ZMB 77479, 77481). This might indicate that the band is present in young males and lost with aging. Variation of webbing formula is **I**2.25-(2.25–2.75)**II**(2^{+}–2.25)-(3–3^{+})**III**2.5-(2^{+}–2.25)**IV** for the hand and **I**(2–2^{+})-(2^{+}–2.5)**II**1.5-3**III**(2–2^{-})-(3–3^{+})**IV**(3–3^{-})-(1.75–2^{-})**V** for the foot. Measurements of the paratypes are provided in Table 14.

Genetics. Comparison of the mitochondrial 16S ribosomal RNA confirmed that *Hyperolius jackie* is not conspecific with any of the described taxa for which homologous information is available. Uncorrected p-distance between *Hyperolius jackie* sp. n. and phylogenetically or geographically close species ranged from 4.5 % to 16.8 % (Table 15). The sequences of the two paratypes differed by 0.4 %, reflecting 3 substitutions in the 487 base pairs. A preliminary phylogenetic analysis showed that *H. jackie* forms a well-supported clade with *H. castaneus* Ahl, 1931, *H. cystocandicans* Richards & Schiøtz, 1977, *H. discodactylus* Ahl, 1931 (and *H. alticola* Ahl, 1931), *H. frontalis* Laurent, 1940, and *H. lateralis* Laurent, 1940 (unpublished data). Relationships within this clade are not well resolved but *H. jackie* appears to be sister to a clade consisting of *H. castaneus*, *H. frontalis*, and *H. cystocandicans* (unpublished data).

Advertisement call. All male type specimens were encountered while calling. Calling started at dusk. The advertisement call of the holotype (ZMB 77476), two paratypes (ZMB 77480 and 77782) and three non-collected specimens were recorded. Air temperature ranged between 15.8 °C and 16.3 °C. The advertisement call consisted of a single pulse group. Usually, a series of calls was emitted, consisting of two to thirteen consecutive calls (Figure 25). The calls were emitted at a rate of 5.54 ± 0.35 (4.81–6.29) per second (mean ± SD [range]) within a series. The pulse group was composed of 10–18 (median: 14) pulses and had a length of 75.4 ± 12.8 (54–107) ms (Figure 25). Pulse rate varied between notes and also within the note between 125 and 250 pulses per second, being highest at the beginning of the note and lowered towards the end. Amplitude modulation was prominent. Relative amplitude rose from the beginning to the middle of the call and declined towards the end.

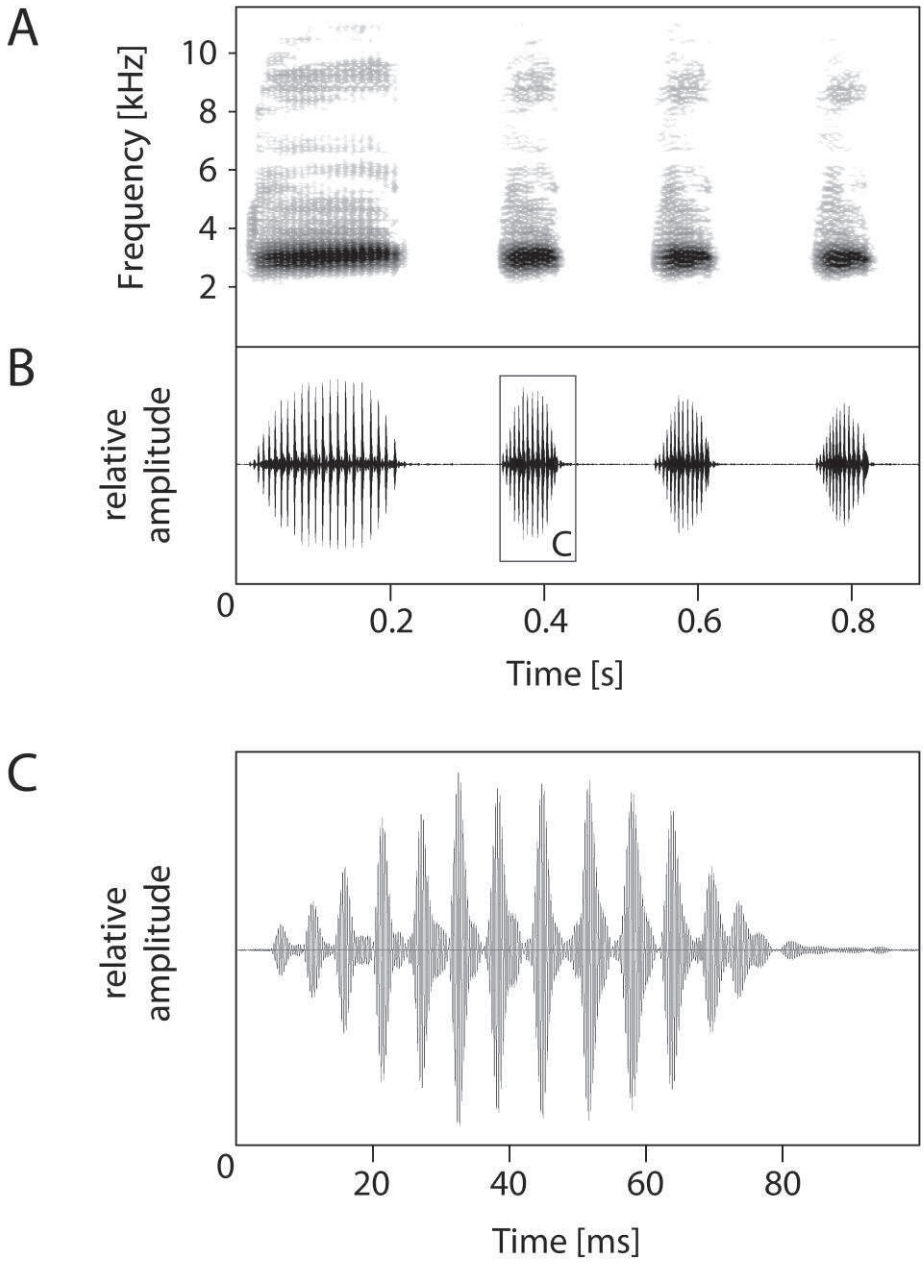

Figure 25. (A) Audiospectrogram and (B) oscillogram of a call series of the holotype of *Hyperolius jackie* sp. n. (ZMB 77476), including an initial note (call type 2) followed by three advertisement calls. (C) Oscillogram of a single advertisement call.

Dominant frequency ranged between 2650 and 3150 Hz (Figure 25). There were prominent harmonics at ca. 6000, 9000, 12000 and 15000 Hz, and less distinct also at 18000 and 21000 Hz. There was a slight frequency modulation with the dominant frequency of the single pulses within a note differing for up to 100 Hz but without an obvious pattern. In most calls, however, the lowest frequencies were measured in the first half of the pulses and the highest in the second half. About two-thirds of the call series were initiated by another call

type which preceded the advertisement calls. It consisted of a single pulse group and generally resembled the advertisement call in structure and dominant frequency but was much longer (180.1 ± 28.7 [130–223] ms), consisted of a higher number of pulses (17–25; median 20), and had a much lower pulse repetition rate (90–166 pulses per second) (Figure 25). This call appeared to be an abbreviated aggression call. Three aggression calls, which were emitted without adding a series of advertisement calls, were recorded and analysed. They were longer (244–341 ms), consisted of 26–35 pulses, and had a pulse repetition rate of 71–142 pulses per second.

Ecology and distribution. Type specimens were collected between 19:00 h and 00:45 h perching on leaves in vegetation (*Rubus steudneri* and *Juncus effusus*), about 1 m above the ground. Air temperatures recorded during collection with males being active ranged from 15.0°C (19 March 2011, 22:30 h) to 18.2°C (20 March 2011, 19:00h). All type specimens were collected at a single locality, a swamp at Karamba in the southwestern part of the Nyungwe National Park (Figure 26). I heard and recorded the characteristic call of the species at another locality at a small stream between the type locality and the edge of the forest (S 2°27'49.81'', E 29°06'02.88'', 1881 m a.s.l.) but did not collected nor see specimens there (Figure 26). The species probably also occurs in similar habitats in other areas of Nyungwe National Park and in the adjacent Kibira National Park in Burundi. I found four frog species

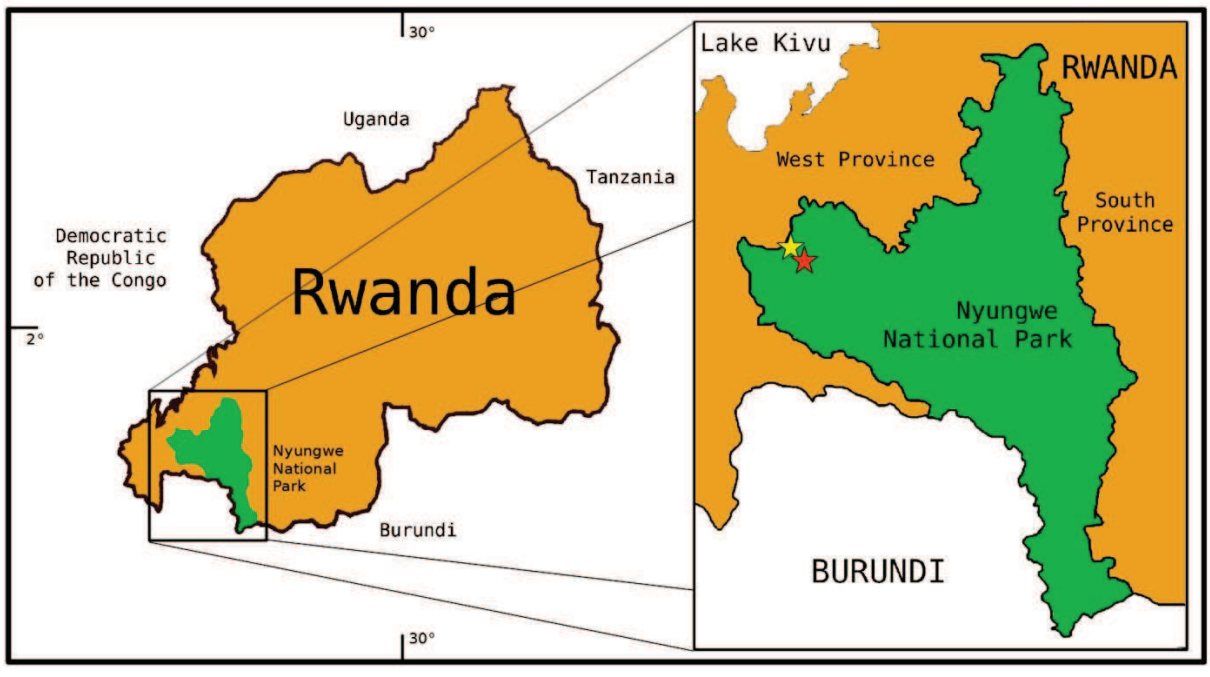

Figure 26. Recording sites of *Hyperolius jackie* sp. n. in Nyungwe National Park, southern Rwanda. Red star: Type locality at Karamba. Yellow star: Second locality at stream near the forest edge.

syntopically with the new species, *Hyperolius castaneus*, *Leptopelis kivuensis* Ahl, 1929, *Amietia* cf. *angolensis* (Bocage, 1866), and *Xenopus wittei* Tinsley, Kobel and Fischberg, 1979. *Phlyctimantis verrucosus* Boulenger, 1912 has also been recorded at this locality (E. Fischer, pers. comm.). At the second site at the stream, several calling males of *Hyperolius discodactylus* were recorded. Apparently*, Hyperolius jackie* is quite sensitive to light. Shortly after I started to look for specimens around the pond at Karamba with a torch, males stopped calling and remained silent even after I turned off the light. In contrast, specimens of *Hyperolius castaneus* kept calling even if the light beam was pointed directly at them from close distance.

Etymology. The species name is dedicated on behalf of Gordon Buchan to Jacqueline Mary Buchan in recognition of support of biodiversity research and nature conservation through the BIOPAT initiative. The name is used as an invariable noun in apposition.

Discussion

Hyperolius jackie is morphologically distinct from all other described species of the genus from Rwanda which occur sympatrically or parapatrically, i.e. *H. castaneus*, *H. discodactylus*, *H. kivuensis* Ahl, 1931, *H. lateralis*, and species of the *H. nasutus* group, *H. cinnamomeoventris* group, and *H. viridiflavus* group. On the basis of the toe webbing which does not reach beyond the distal subarticular tubercle on the postaxial side of Toe III, not or only slightly beyond the distal subarticular tubercle on the preaxial side of Toe V and beyond the penultimate tubercle of Toe IV in *Hyperolius jackie*, the new species can be distinguished from *H. castaneus*, *H.* cf. *cinnamomeoventris*, *H. discodactylus*, *H. kivuensis*, *H. lateralis*, *H.* cf. *nasutus*, and species of the *H. viridiflavus* complex, in all of which the webbing reaches at least halfway between the distal subarticular tubercles of Toes III and V and between the penultimate and distal tubercle of Toe IV. The tibiotarsal articulation reaches to the level of the nostril in *H. jackie* and thus, its legs are relatively longer than those of *H. castaneus*, *H.* cf. *cinnamomeoventris*, *H. kivuensis*, and *H. lateralis* in all of which the tibio-tarsal articulation reaches to the level of the eye only. The translucent ventral skin distinguishes *H. jackie* from all other Rwandan species of the genus except *H. discodactylus*. Furthermore, *H. jackie* (characters in parentheses) differs from the Rwandan species in the following morphological

characters: In *H. castaneus*, the dorsum is light brown with dark brown band from tip of snout along canthus rostralis, continued as dorsolateral band to groin (vs. dorsum light green without any or with a faint whitish dorsolateral stripe), foot and lateral faces of legs are red in life (vs. yellowish green), the iris is greyish-brown (vs. coppery), adult males are larger (SUL 21.2–26.9 mm, mean: 24.3 mm, n=32; vs. 18.8–23.2 mm, mean 20.8 mm, n=8), and have conspicuous pectoral and brachial glands (vs. absent). In males of *H. discodactylus*, the dorsum is coarsely granular with several large keratinous black tubercles (vs. dorsum smooth with few tiny tubercles), the snout is rounded in dorsal view (vs. acuminate), and adult males are much larger (SUL 29.4–34.4 mm, mean 32.1, n= 5; vs. 18.8–23.2 mm, mean 20.8 mm, n=8) and have conspicuous pectoral and brachial glands (vs. absent). Males of *H.* cf. *cinnamomeoventris* have a yellowish-brown to light brown dorsum with a conspicuous yellow stripe running from the tip of the snout along the canthus rostralis, the distal edge of the upper eyelid, the dorsolateral side of the trunk to the groin (vs. dorsum light green without any or with a faint whitish dorsolateral stripe), and red blotches are usually present on the lateral faces of the thigh (vs. absent). In *H. kivuensis*, the dorsum is brown or dark green with a distinct broad black band running dorsolaterally from the tip of the snout to the groin (vs. dorsum light green without any or with a faint whitish dorsolateral stripe), the snout is pointed in dorsal view (vs. acuminate), adult males are much larger (SUL 23.5–33.9 mm, mean 28.4 mm, n=14; vs. 18.8–23.2 mm, mean 20.8 mm, n=8), and the iris is greyish-brown (vs. coppery). In *H. lateralis*, the dorsum is either light brown to cream-coloured with a light, yellowish dorsolateral stripe or green with a broad white lateral band (vs. dorsum light green without any or with a faint whitish dorsolateral stripe), the lateral sides of the thigh and the groin usually have red blotches or speckling (vs. absent), and the iris is greyish-brown (vs. coppery). In the Rwandan species of the *H. nasutus* group, the body is very elongate and the head relatively narrower (HW/SUL 0.28–0.31, n=12; vs. 0.36–0.37, n=8), the snout is pointed (vs. acuminate), and males are smaller (SUL 18.6–20.2 mm, mean 19.4 mm, n=10; vs. 18.8–23.2 mm, mean 20.8 mm, n=8). Adult males of the species of the *H. viridiflavus* complex from Rwanda are much larger (SUL 25.2–33.7, mean 29.4, n=16; vs. 18.8–23.2 mm, mean 20.8 mm, n=8), the dorsal colouration is extremely variable with the basic colouration ranging from cream-coloured, yellow or light brown to reddish brown, dark brown and almost black but is never light green like in *H. jackie*.

According to the results of the genetic analysis, *Hyperolius jackie* is relatively closely related

to two species which are not known to occur in Rwanda, *H. frontalis*, and *H. cystocandicans* (Table 15). In *Hyperolius frontalis*, the dorsum is green like in *H. jackie* but the snout has a white or yellowish triangulate blotch which is continued as an interrupted dorsolateral band of blotches from the eye halfway to the groin (vs. absent), the dorsal surfaces are shagreened with larger tubercles (vs. smooth with few tiny tubercles), the snout is rounded (vs. acuminate), the toe webbing is more developed, reaching to distal subarticular tubercle on Toe IV (vs. less developed, reaching to penultimate tubercle only), and males are larger (SUL 25–29 mm vs. 18.8–23.2 mm). Males of *H. cystocandicans* are larger (SUL 23–28 mm vs. 18.8–23.2 mm), have pectoral and brachial glands (vs. absent), a yellowish to light brown dorsum (vs. light green), and the toe webbing is more developed, reaching to distal subarticular tubercle on Toe IV (vs. less developed, reaching to penultimate tubercle only).

A number of species have been described from the montane or submontane areas of the Albertine Rift in eastern DRC and western Uganda and are not known to occur in Rwanda. Most of these species (e.g. *H. balfouri* [Werner, 1907], *H. langi* Noble, 1924, *H. leleupi* Laurent, 1951, *H. ferrugineus* Laurent, 1943, *H. xenorhinus* Laurent, 1972) can easily be distinguished from *H. jackie* based on the size of adult males and dorsal and ventral colouration (Laurent 1950a, 1951, 1972a, Schiøtz 1999). Three species, however, appear to be similar to *H. jackie* based on their original descriptions, i.e. *H. chrysogaster* Laurent, 1950, *H. diaphanus* Laurent, 1972, and *H. leucotaenius* Laurent, 1950. Examination of the available type material of these species combined with data from the original descriptions revealed the following differences (characters of *H. jackie* given in parentheses): In males of *H. chrysogaster*, the head is narrower with HW/SUL 0.30–0.32 (vs. 0.36–0.37), the heels overlap considerably when the knees are flexed and thighs are held vertically to median plane (vs. heels overlapping each other slightly), the webbing is considerably more developed, formula being I2-2II2--3-III2-2IV for the hand (vs. **I**2.25-[2.25–2.75]**II**[2^+–2.25]-[3–3^+]**III**2.5-[2^+–2.25]**IV**) and **I**(1.5–2^-)-2^+**II**1-(2^+–2.5)**III**1.5-(2–2.5)**IV**2-(1^+–1.25)**V** for the foot (vs. **I**[2–2^+]-[2^+–2.5]**II**1.5-3**III**[2–2^-]-[3–3^+]**IV**[3–3^-]-[1.75–2^-]**V**), size is smaller (17.5–19.5 mm, mean 18.6 mm, n=5; vs. 18.8–23.2 mm, mean 20.8 mm, n=8), a skin flap is present above the vent (vs. absent), and conspicuous pectoral and brachial glands are present (vs. absent).

Hyperolius diaphanus is very similar to *H. frontalis* according to the original description. Males of *H. diaphanus* differ from those of *H. jackie* in a larger size (SUL 21.6–27.5 mm,

mean 25.4 mm, n=26; vs. 18.8–23.2 mm, mean 20.8 mm, n=8), the webbing is considerably more developed, formula being **I**2-2**II**2-3^{-}**III**2-2**IV** for the hand (vs. **I**2.25-[2.25–2.75]**II**[2^{+}–2.25]-[3–3^{+}]**III**2.5-[2^{+}–2.25]**IV**) and **I**1.5-2**II**1-2**III**1^{+}-2**IV**2-1**V** for the foot (vs. **I**[2–2^{+}]-[2^{+}–2.5]**II**1.5-3**III**[2–2^{-}]-[3–3^{+}]**IV**[3–3^{-}]-[1.75–2^{-}]**V**), and the iris is silver with some golden shimmer in life (vs. coppery).

Males of *Hyperolius leucotaenius* have conspicuous pectoral and brachial glands (vs. absent), are light green dorsally like *H. jackie* but have a conspicuous white band that is bordered with black running from the tip of the snout along canthus rostralis, distal edge of upper eyelid, dorsolateral side of trunk to the groin (vs. canthus rostralis and edge of upper eyelid speckled with brown, dark-bordered light dorsolateral band absent), the extremities are red (vs. yellowish green), and the toes are webbed beyond the penultimate subarticular tubercle of Toe IV and the distal subarticular tubercles of Toes III and V (vs. not beyond these tubercles).

Hyperolius jackie appears to be closely related to *H. castaneus*, *H. cystocandicans*, *H. discodactylus*, *H. frontalis*, and *H. lateralis*. The p-distances in the 16S rRNA sequence between *H. jackie* and these species are 4.5–7.5 % (Table 15) which is within the range observed between all the six species (3.8–8.0 %). The new species appears to be most closely related to *H. castaneus* with which it occurs syntopically in the Karamba swamp in Nyungwe National Park and from which it differs by a p-distance of 4.5 % (Table 15). This value is higher than that between the well distinguished species *H. castaneus* and *H. frontalis* (4.3 %) and *H. discodactylus* and *H. lateralis* (3.8 %) (unpubl. data).

The close relationship that is suggested by the results of the analysis of the 16S rRNA gene, is supported by the structure of the advertisement call of *Hyperolius jackie* which is similar to those of *Hyperolius castaneus*, *H. cystocandicans*, *H. discodactylus*, *H. frontalis*, and *H. lateralis* all of which have short rasping calls consisting of a single pulse group (Schiøtz 1999, Köhler *et al*. 2005a, Sinsch *et al*. 2011, unpubl. data). These call properties distinguish these species from all other *Hyperolius* species from Rwanda whose advertisement call are a single, unpulsed note (*H. viridiflavus* group) or consist of a short pulse group (“buzzing note”) followed by three short pulses (*H.* cf. *nasutus*), one to three short clicks (*H.* cf. *cinnamomeoventris*), or of a pulse group with few pulses of varying interpulse intervals (*H. kivuensis*) (Sinsch *et al*. in press, unpubl. data). The call of *H. jackie* has a higher dominant

frequency (2650–3150 Hz) than the call of *H. discodactylus* (2000–2500 Hz) and a lower one than the call of *H. lateralis* (3100–3800 Hz). The call is shorter (54–107 ms) and consists of less pulses (10–18) than the call of *H. discodactylus* (195–320 ms, 31–53 pulses) and is longer and has more pulses than the call of *H. castaneus* (38–52 ms, 7–10 pulses) and *H. lateralis* (13–42 ms, 4–8 pulses). Unlike the calls of *H. castaneus*, *H. cystocandicans*, and *H. lateralis* which are emitted almost continuously at a relatively low rate, the calls of *H. jackie* are emitted in distinct call series at a high rate. The 2–13 calls within a call series are emitted at a rate of 4.81–6.29 calls per second which distinguish the call series from those of *H. discodactylus* (1–4 calls, 0.72–0.85 calls per second) and *H. frontalis* (2–3 calls, ca. 3 calls per second).

Two remarkable characters of *Hyperolius jackie* are the transparent ventral skin and the blue colouration of the bones. However, these features are not unique to this species. Its aforementioned closely related congeners also have a more (*H. discodactylus*, *H. frontalis*) or less (*H. castaneus*, *H. cystocandicans*, *H. lateralis*) transparent ventral skin (pers. observ.). The bluish colouration of the bone can also be observed at least in *H. castaneus*, *H. discodactylus* and *H. lateralis* (unpubl. data).

The discovery of a new, apparently endemic frog underlines the importance of the Nyungwe National Park as a protected area. The lower montane forest of Nyungwe National Park continues into the Kibira National Park in Burundi and is one of the largest forests of its kind in Africa. It is renowned for its species richness, and 1105 species of plants, among them 280 Albertine Rift endemics and 47 local endemics, as well as 13 species of primates and 285 species of birds have been reported from the park (Plumptre *et al*. 2007, Fischer & Killmann 2008).

Hyperolius jackie is so far known only from the Karamba swamp and a neighbouring stream in Nyungwe National Park, and thus, has to be regarded as endemic to this area. Five other species occur syntopically at this location, i.e. *Leptopelis kivuensis*, *Amietia* cf. *angolensis*, *Phlyctimantis verrucosus*, *Xenopus wittei*, and *Hyperolius castaneus*, with the latter being the most common and most easily detectable species. This confirms the concept of Sinsch *et al*. (2011) who consider *H. castaneus* an umbrella species suited for the identification of important breeding sites for Albertine Rift endemic amphibians.

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VI

Taxonomy of the super-cryptic *Hyperolius nasutus* group of long reed frogs of Africa (Anura: Hyperoliidae), with descriptions of six new species

This chapter has been published as:

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Abstract

Specimens from across the range of the *Hyperolius nasutus* species group were sequenced for two mitochondrial genes and one nuclear gene. Advertisement calls were recorded from the same specimens where possible and morphological characters were compared. Bayesian inference and maximum likelihood produced a tree indicating 16 clades. The clades show little or no overlap in combinations of 16S sequence difference, shared tyr haplotypes, advertisement call parameters, snout profiles and webbing. On the basis of these data we recognise *H. acuticeps*, *H. adspersus*, *H. benguellensis*, *H. dartevellei*, *H. igbettensis*, *H. nasutus*, *H. nasicus*, *H. poweri*, *H. viridis* and describe six new species: *Hyperolius friedemanni* sp. nov. Mercurio & Rödel, *Hyperolius howelli* sp. nov. du Preez & Channing, *Hyperolius inyangae* sp. nov. Channing, *Hyperolious jacobseni* sp. nov. Channing, *Hyperolius rwandae* sp. nov. Dehling, Sinsch, Rödel & Channing, and *Hyperolius lupiroensis* sp. nov. Channing. *Hyperolius lamottei* is confirmed to be outside the *H. nasutus* group clade. *Hyperolius granulatus*, *H. oxyrhynchus*, *H. punctulatus* and *H. sagitta* are assigned as junior synonyms. As our results are based on a small number of specimens, these hypotheses await testing with larger sample sizes and more characters. A species distribution model suggests where outlier populations might be found.

Key words: *Hyperolius nasutus* group, new species, phylogeny, taxonomy, advertisement calls, biogeography, molecular genetics, morphology, species distribution model

Introduction

African reed frogs in the genus *Hyperolius* Rapp, 1842 are highly speciose, with 128 species currently recognised (Frost 2011). Many of them are brightly patterned and polymorphic (Schiøtz 1975, 1999), but all are poor in external diagnostic morphological characters, making it difficult to identify preserved material. Many of the original descriptions are not diagnostic which makes it difficult to assign names reliably. The advent of sound analysis that had become popular from the 1960s allowed the use of this non-morphological technique to identify *Hyperolius* species in the field (Schiøtz 1975; Köhler *et al.* 2005a; Dehling 2012a). More recent phylogenetic studies have used DNA to help delimit *Hyperolius* species (Rödel

et al. 2010; Schick *et al.* 2010; Conradie *et al.* 2012; Dehling 2012a), and to even identify cryptic genera (Rödel *et al.* 2009). These studies are examples of an approach that has led to a huge increase in the number of amphibian species recognised worldwide (Köhler *et al.* 2005b). Mercurio (2011) provides illustrations and calls of some cryptic *Hyperolius* species.

Within *Hyperolius*, there are a number of proposed species groups (Schiøtz 1975). One of these, the long reed frogs in the *Hyperolius nasutus* group is widespread in sub-Saharan Africa. Recent molecular work revealed that these frogs are basal in a clade of some *Hyperolius* species (Rödel *et al.* 2009; Veith *et al.* 2009). Within this group many species have been described, and currently 15 species names are available (Amiet 2005). Channing *et al.* (2002) recognised three different advertisement call types across the range of the complex, and suggested that these represented three species (*H. nasutus*, *H. acuticeps*, *H. viridis*), with others regarded as incertae sedis as no calls were known for them. They proposed *H. lamottei* to be a junior synonym of *H. nasutus*, on the basis of a similar advertisement call. In addition they confirmed *H. viridis* as valid, based on calls and material from near Sumbawanga in Tanzania.

Schiøtz & van Daele (2003) identified two species in north-western Zambia, using advertisement calls, which they referred to *H. nasutus* and *H. benguellensis*, in contrast to Channing *et al.* (2002) who had assigned two call types from the same locality to *H. acuticeps* and *H. nasutus*.

In Cameroon, two species were recognised by Amiet (2005), *H. adspersus* and *H. igbettensis*, which he distinguished using morphological features such as the snout shape and differences in webbing. There were also two species tentatively recognised in central Democratic Republic of Congo (hereafter DRC) (Schiøtz 2006a), *H. adspersus* and *H. nasicus* with a sharp snout tip.

Recently, Schiøtz (2006b) reviewed the state of the taxonomy of the group. He noted that the various characters used to separate the species often delimited different sets of specimens. His main conclusions were that *H. lamottei* was not part of the *H. nasutus* group, based on consistent call and colour pattern differences. He showed that the specimens that Channing *et al.* (2002) collected near Sumbawanga in Tanzania and identified as *H. viridis* (based on advertisement call), were actually not 'true' *H. viridis*, but other members of the *H. nasutus* group.

In the molecular hyperoliid phylogeny of Veith *et al.* (2009), having a limited sample size of long reed frogs at hand, three well supported lineages (of one specimen each) were evident, which the authors referred to as *H. acuticeps* (Kenya) and *H. nasutus* complex A (Ivory Coast) and B (Namibia). In a tree based on mitochondrial (mt) and nuclear markers, the single representative of long reed frog (*H. nasutus* complex B) appeared as a sister taxon to *Morerella*, which could neither be confirmed nor rejected by Rödel *et al.* (2009). As only 25% of *Hyperolius* species were included in their analysis, the position of the *Hyperolius nasutus* clade is not yet confirmed.

The IUCN recognises eight species in the group (IUCN 2011): *Hyperolius acuticeps* Ahl, 1931 (type locality Konde-Nyika, = Poroto Mts, Tanzania) extending from northern coastal South Africa to Ethiopia, including much of Mozambique, Zimbabwe, Zambia, Malawi, Tanzania, south-eastern DRC, Burundi, Rwanda, Kenya, Uganda and south-eastern Sudan (Amiet 2006b); *Hyperolius adspersus* Peters, 1877 (type locality Chinchoxo, Cabinda, Angola) distributed from central Cameroon south to the Congo River (Schiøtz 2006b); *Hyperolius benguellensis* (Bocage, 1893) (type locality Caota, Angola) overlaps both *H. nasutus* and *H. acuticeps* in Angola, Zambia, Zimbabwe, northern Mozambique and south-eastern DRC (Schiøtz & Poynton 2008); *Hyperolius igbettensis* Schiøtz, 1963 (type locality Igbetti, Nigeria) distributed from the savannas of the Ivory Coast east to central Cameroon (Schiøtz *et al.* 2008a); *Hyperolius lamottei* Laurent, 1958 (type locality Mt Nimba area) known from Ivory Coast to southern Senegal (Rödel & Schiøtz 2004); *Hyperolius nasicus* Laurent, 1943 (type locality Kasiki, DRC) only known from the type material (Schiøtz 2006b); *Hyperolius nasutus* Günther, 1865 “1864” (type locality Calandula, Angola) covering Angola but extending north into the Congo Basin, east into western Zambia and south into northern Namibia and the Okavango Swamps of Botswana (Amiet 2006a); *Hyperolius viridis* Schiøtz, 1975 (type locality near Sumbawanga, Tanzania) from a restricted area in Tanzania, possibly extending into adjacent countries (Schiøtz 2006b).

The current debate has raised a number of questions. In the framework of a taxonomic review, we attempt to answer them, based largely on molecular data. These include: Is *H. lamottei* a distinct species? Is *H. lamottei* part of the *H. nasutus* clade? Can the species outlined above be confirmed using molecular data? Are the disputed species assignments of Channing *et al.* (2002) resolved? Are there unrecognised cryptic species?

The confusion due to colour polymorphism and the paucity of calls and DNA sequences from material referable to types has considerably muddied the waters of this ubiquitous species complex. Where two or more species of the group may be sympatric, it is essential to record calls and take tissue samples from the same voucher specimen. This molecular project was initiated to provide a testable hypothesis of species boundaries and provide a framework for future studies, as more data become available.

Material and methods

Approach

We use the accepted species assemblage that makes up the *Hyperolius nasutus* group (IUCN 2011; Schiøtz 1999). Our approach was to sequence fragments of two mitochondrial genes (12S, 16S) and to use the sequence information to identify potential species. A phylogeny was constructed and we included genetic distances calculated for the 16S fragment. In amphibians, this is a widely accepted marker in DNA bar-coding (e.g. Vences *et al.* 2005). We also sequenced 40 specimens for the nuclear gene Tyrosinase exon 1, separated into the most likely haplotype phases as explained below. Once the species boundaries were hypothesised, the sequenced specimens were examined for advertisement calls and morphological characters that are elsewhere considered useful to recognize long reed frog taxa, such as snout shape and general body proportions (see Amiet 2005). The discovered clades were then compared to the type descriptions and the type specimens, in order to match the available names to the suggested species clades as defined by the molecular study. Specimens for which only a single gene sequence, for example 12S, was available, were not included in the initial clade recognition. They were subsequently assigned to the discovered clades based on close sequence similarity. Advertisement calls, most recorded from the same specimens that were sequenced, were analysed (see below). These specimens provide a positive link between gene sequence, morphology and advertisement call, as proposed by Channing *et al.* (2002).

Sampling

Tissue samples were obtained from field-collected specimens and preserved in 95% ethanol. Usually more than one specimen per locality was sampled. All available GenBank

(http://www.ncbi.nlm.nih.gov/) 12S and 16S sequence pairs from the group were checked and incorporated, and unpublished sequences were kindly made available by A. van der Meijden and M. Vences. Samples for which sequence data are available were collected from Angola, Botswana, Central African Republic, DRC, Congo-Brazzaville, Gabon, Ghana, Guinea, Ivory Coast, Kenya, Malawi, Mozambique, Namibia, Rwanda, South Africa, Sierra Leone, Tanzania, Zambia and Zimbabwe. The outgroup (*Hyperolius angolensis*) was represented by a specimen from Humpata, Angola (PEM A10106), GenBank accession number JQ513623. Additional specimens and/or tissues were generously provided by the United States National Museum, Washington (USNM), Museum of Comparative Zoology, Harvard (MCZ), Copenhagen Natural History Museum (ZMUC), the South African Institute of Aquatic Biodiversity, Grahamstown (SAIAB), E. Netherlands (EN), J. Harvey (JH) and N. Jacobsen (NJ). Type material was examined from the Natural History Museum, London (NHM), the Museum für Naturkunde, Berlin (ZMB) and the Royal Museum for Central Africa, Tervuren (RMCA). Photographs of the type of *Hyperolius poweri* were made available by the MCZ. Call recordings were made available through the courtesy of ZMUC, NJ and Colin Tilbury. Voucher specimens collected as part of this project have been deposited at ZMB, Port Elizabeth Museum (PEM), National Museums of Kenya, Nairobi (NMK) and SAIAB. Field numbers include LdP (L. du Preez), AC (A. Channing), LOM and MTN (A. Hillers).

Morphology

These small frogs have few useful features for diagnosing species. The snout shapes have been used, but these show a surprising range of variation (Amiet 2005). Snout shape in lateral view can be rounded, angular or angular with a protruding tip. Poynton (1964:193) illustrates the need to understand variation and the difficulty of using morphological characters to separate species in this group. A detailed discussion was presented in Poynton & Broadley (1987).

We used the following characters and abbreviations which have been used in previous work (Poynton 1964; Schiøtz 1975; Amiet 2005; Dehling 2012a): snout-urostyle-length (SUL, from tip of snout to posterior end of urostyle); tibiofibula-length (TFL, measured by both knee and tibio-tarsal articulation flexed); total leg length (LEG, from vent to tip of fourth toe with leg fully extended at right angle to body axis); foot length (FOT, from proximal end of inner metatarsal tubercle to tip of fourth toe); hand length (HND, from proximal end of thenar

tubercle to tip of third finger); head width (HW, measured at corners of the mouth); head length (HL, from posterior end of mandible to tip of snout); eye diameter (ED, horizontal diameter of the eye); eye-to-nostril distance (EN, from anterior edge of orbit to centre of nostril); nostril-snout distance (NS, from centre of nostril to tip of snout); internarial distance (NN, distance between centres of nostril); snout length (SL, from anterior edge of orbit to tip of snout); eye distance (EE, distance between anterior edges of eyes); interorbital distance (IO, shortest distance between upper eyelids); upper eyelid width (EW, maximum width of upper eyelid); length of thigh (THL). All measurements are given in mm. Webbing was illustrated using a diagram, following Biju *et al.* (2011); snout shape was described; and the dorsal pattern elements were noted.

Advertisement call

Calls were recorded in the field, and analysed using RavenPro 1.4 with the following settings: Hann type spectrogram, with a DFT size of 128, and a 50 % time grid overlap.

DNA extraction and sequencing

Tissues were digested using standard Proteinase-K protocol, and DNA was extracted using phenol-chloroform (Hillis *et al.* 1996). A 550 bp fragment of the mt 16S gene was amplified using the primers 16SaR-F and 16SbR-R of Kocher *et al.* (1989), as modified by Bossuyt & Milinkovitch (2000) annealing at 51°C, and a 450 bp fragment of the mt 12S gene using the primers 12SA-F and 12SB-R (Goebel *et al.* 1999), annealing at 56 °C. A 530 bp fragment of the nuclear tyrosinase exon 1 (Bossuyt & Milinkovitch 2000), annealing at 55 °C, was sequenced for one or more representatives of each species. We used Fast Taq readymix (Kapa Biosystems) for PCR, using the manufacturer's recommended protocol; an initial denaturing step of 1 minute at 95°C, followed by 35 cycles of denaturing for 10 seconds at 95°C, annealing for 10 seconds, extension for 1 second at 72°C. There is no final extension step. Primer sequences are given in Table 16.

Table 16. Primer sequences used in this study.

Name and source	Sequence (5' to 3')
12SA-F (Goebel *et al.* 1999)	AAACTGGGATTAGATACCCCACTAT
12SB-R (Goebel *et al.* 1999)	GAGGGTGACGGGCGGTGTGT
16SaR-F (Bossuyt & Milinkovitch 2000)	CGCCTGTTTAYCAAAAACAT
16SbR-R (Bossuyt & Milinkovitch 2000)	CCGGTYTGAACTCAGATCAYGT
TyrC-F (Bossuyt & Milinkovitch 2000)	GGCAGAGGAWCRTGCCAAGATGT
TyrG-R (Bossuyt & Milinkovitch 2000)	TGCTGGCRTCTCTCCARTCCCA

Sequencing reactions and electrophoresis were carried out by the University of Stellenbosch Central Analytical Facility. Forward and reverse strands were sequenced for all samples. Both sequences were checked against the chromatograms, trimmed, and combined into a single contig for each fragment using Sequencher 5.1 (GeneCodes Corporation). All mt and nuclear sequences were checked using BLAST to confirm their placement in the ingroup (http://blast.ncbi.nlm.nih.gov/). All new sequences were deposited in GenBank (Benson *et al.* 2012). Appendix 7 is a gazetteer of the collection localities. The nuclear tyr gene was phased into the most likely two haplotypes for each individual by first submitting the edited sequences including IUPAC polymorphism symbols, to SeqPhase step 1 (Flot 2010) an online service that prepares a simplified output file. The output from SeqPhase is then used as input to PHASE (Stephens & Donnelly 2003), which computes the likelihood of possible haplotypes. The output from PHASE is converted to full sequences through SeqPhase step 2.

Molecular analysis

Sequences were trimmed and concatenated using Sequencher 5.1, then aligned using Clustal W2 (2.0.12) with default settings. JModelTest was used to determine the appropriate model of evolution under AIC. The aligned sequences were input into MrBayes 3.2.1, and run for 10 million generations, with three attempted swaps each iteration, with the temperature set at 0.1, and using the GTR + G + I model. Two independent runs were analysed, each with one hot and three cold chains. The data were partitioned into three separate gene fragments (12S, 16S and tyr), and each partition was treated independently. The default burn-in value of 25% was used. A second analysis only using all available 16S sequence data was analysed in a similar manner. Maximum likelihood (ML) models were analysed using Garli 2.0. Bootstrap support was determined using 1000 bootstrap repetitions each with three search repetitions. Uncorrected 16S sequence divergence was determined using PAUP*. A haplotype network was constructed for the tyr sequences, using TCS 1.21 (Clement *et al.* 2000) that implements statistical parsimony to estimate gene genealogies (Templeton *et al.* 1992).

Species distribution modelling

The potential distribution of the whole *nasutus* group was assessed within a GIS-based analysis using the CRAN-R package 'dismo' (version 07.17; Hijmans *et al.* 2012). We obtained information on long-term climatic conditions within sub-Saharan Africa from the Worldclim

database as interpolated average conditions within the time period 1950–2000 (Hijmans *et al.* 2005). Nineteen bioclimatic variables (Nix 1986) at all available records of long reed frog localities were extracted and a BIOCLIM (Nix 1986) model was computed. This model describes the environmental space occupied by all available species records of the group shown in Figure 27.

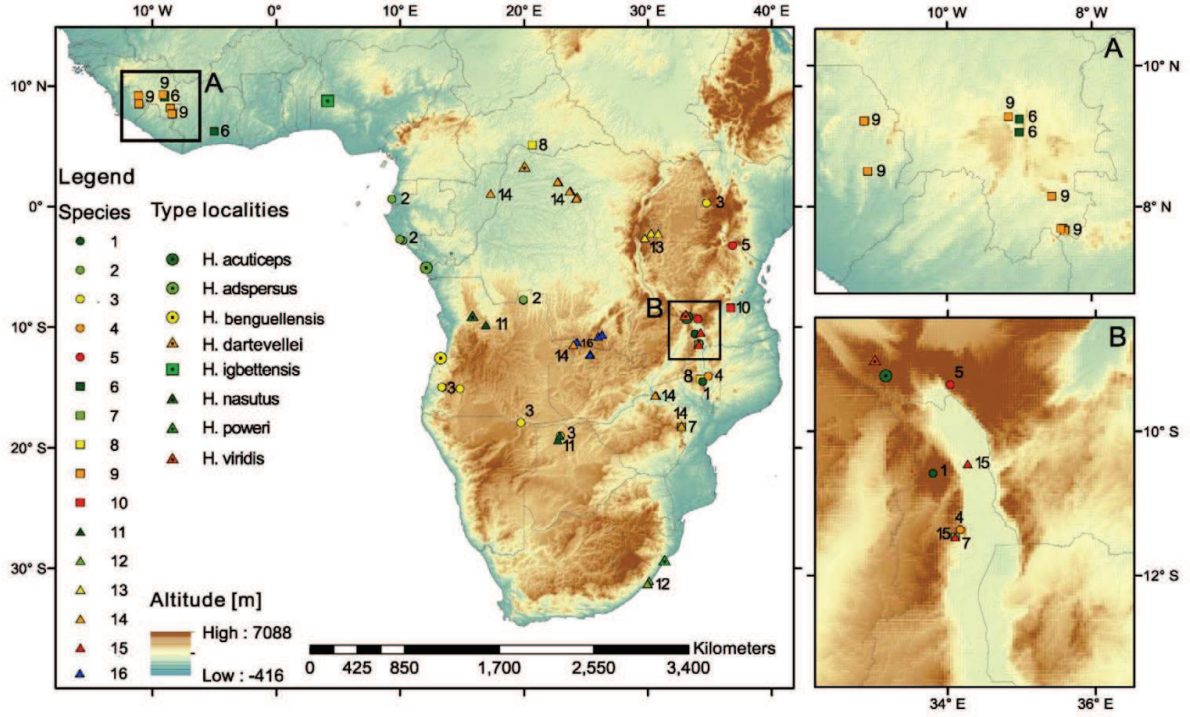

Figure 27. Map showing the distribution of the sequenced specimens of recognised species in the *Hyperolius nasutus* group plus *H. lamottei*. Species numbers represent the following: (1) *H. acuticeps*, (2) *H. adspersus*, (3) *H. benguellensis*, (4) *H. friedemanni* sp. nov., (5) *H. howelli* sp. nov., (6) *H. igbettensis*, (7) *H. inyangae* sp. nov., (8) *H. jacobseni* sp. nov., (9) *H. lamottei*, (10) *H. lupiroensis* sp. nov., (11) *H. nasutus*, (12) *H. poweri*, (13) *H. rwandae* sp. nov., (14) *H. dartevellei*, (15) *H. viridis*, (16) *H. nasicus*.

Results

The available genetic material and specimens are listed under the relevant species below. Most tissue samples were accompanied by voucher specimens, while a few were not vouchered, although these were usually duplicates of voucher specimens, which had been toe-clipped in the field and released. The DNA sequences are accessioned in GenBank, (JQ863547–JQ863780; KC409065–KC409087). The 131 samples resolve into 16 terminal groups in a phylogeny based on 16S sequences. The arrangement is congruent with a smaller

Table 17. Summary of the differences between the species, in the form: 16S/tyr/call/snout/webbing. For 16S the range of differences is given; 'tyr' indicates that no tyrosine exon 1 haplotypes are shared, but is absent where a haplotype is shared; no overlap in advertisement call parameters is indicated by D (duration), P1 (only phase 1 present in one of the pair), P1N (number of pulses in phase 1), P2N (number of pulses in phase 1), P1R (pulse rate of phase 1), P2R (pulse rate of phase 2); difference in snout profile is indicated by SP; difference in webbing is indicated by W.

Abbreviations: Ac—H. acuticeps, Ad—H. adspersus, Be—H. benguellensis, Da—H. dartevellei, Fr—H. friedemanni **sp. nov.**, Ho—H. howelli **sp. nov.**, Ig—H. igbettensis, In—H. inyangae **sp. nov.**, Ja—H. jacobseni **sp. nov.**, Lu—H. lupiroensis **sp. nov.**, La—H. lamottei, Na—H. nasutus, Ni—H. nasicus, Po—H. poweri, Rw—H. rwandae **sp. nov.**, Vi—H. viridis.

	Ac	Ad	Be	Da	Fr	Ho	Ig	In	Ja	La	Lu	Na	Ni	Po	Rw
Ad	16S: 2.1–2.9/tyr/D, P1/SP/W														
Be	16S: 6.0–6.2/tyr/P2R/ SP/ W	16S: 4.2–4.8/tyr/D, P1/ SP/ W													
Da	16S: 2.3–3.5//P1/ SP/ W	16S: 1.4–2.5/tyr/D, P1N// W	16S: 3.9–4.8/tyr/D, P1, P1R/ SP/ W												
Fr	16S: 2.5–3.5//D, P2R/ SP/ W	16S: 1.4–2.5/tyr/D, P1, P1R/ SP/ W	16S: 4.8–5.1/tyr/D, P2R/ SP/ W	16S: 1.5–2.5//D, P1, P1R/ SP/ W											
Ho	16S: 2.7–4.1//D, P1R, P2R// W	16S: 1.6–3.3/tyr/D, P1, P1R/ SP/ W	16S: 5.2–5.4/tyr/D, P2N/ SP/ W	16S: 1.2–2.7//D, P1, P1R/ SP/ W	16S: 1.6–2.7//P1R, P2R/ SP/ W										
Ig	16S: 5.2–5.8// D, P1R, P2R/ SP/ W	16S: 3.5–4.6/tyr/D, P1, P1R/ SP/ W	16S: 6.0–6.2/tyr/D, P1R, P2N/ SP/ W	16S: 3.9–5.2//D, P1, P1R/ SP/ W	16S: 4.6–5.0//P1R/ SP/ W	16S: 4.3-5.6//P1R, P2R/ SP/ W									
In	16S: 4.5–5.2/tyr// SP/ W	16S: 3.3–4.2/tyr/D, P1, P1R/ SP/ W	16S: 4.0–4.8/tyr/D, P2N/ SP/ W	16S: 3.5–4.7/tyr/D, P1, P1R/ SP/ W	16S: 3.9–4.4/tyr/D, P1N, P2R/ SP/ W	16S: 4.3–5.4/tyr/P1N, P2N/ SP/ W	16S: 5.6–6.4/tyr/P1N, P2R/ SP/ W								

……continued on the next page

Table 17. (Continued)

	Ac	Ad	Be	Da	Fr	Ho	Ig	In	Ja	La	Lu	Na	Ni	Po	Rw
Ja	16S: 3.1–3.7/tyr/D, P1, P1R/ SP/ W	16S: 2.1–2.9/tyr/D, P1R///	16S: 5.4/tyr/D, P1, P1R/ SP/ W	16S: 2.7–3.5/tyr/D, P1R/ SP/ W	16S: 2.7–3.1/tyr/D, P1, P1R/ SP/ W	16S: 3.1–3.7/tyr/D, P1, P1R// W	16S: 4.6/tyr/P1, P1R// W	16S: 5.0–5.2/tyr/D, P1, P1R/ SP/ W							
La	16S: 13.1–14.4/tyr/D, P1, P1R/ SP/ W	16S: 13.2–13.9/tyr/D, PIN/ SP/ W	16S: 14.2–14.8/tyr/D, P1, P1R/ SP/ W	16S: 10.9–13.4/tyr/D, P1N/ SP/ W	16S: 13.3–14.0/tyr/D, P1, P1R// W	16S: 13.8–15.0/tyr/D, P1, P1R/ SP//	16S: 13.4–14.0/tyr/D, P1, P1R/ SP/ W	16S: 13.1–15.1/tyr/D, P1, P1R/ SP/ W	16S: 14.5–15.1/tyr/P1 N, P1R/ SP/ W						
Lu	16S: 12.0–12.2/tyr/D, P1, P1R/ SP/ W	16S: 11.4–11.8/tyr/D, P1N// W	16S: 11.7/tyr/D, P1, P1R/ SP/ W	16S: 10.9–11.6/tyr// SP/ W	16S: 11.1–11.6/tyr/D, P1, P1R/ SP/ W	16S: 12.0–12.8/tyr/D, P1R/ SP/ W	16S: 13.5/tyr/D, P1, P1R// W	16S: 11.9–12.0/tyr/D, P1, P1R/ SP/ W	16S: 12.2/tyr/P1 R// W	16S: 13.6–14.0/tyr// SP/ W					
Na	16S: 2.5–2.8/tyr/D, P2R/ SP/ W	16S: 1.2–2.1/tyr/D, P1R// W	16S: 4.1–4.6/tyr/D, P2R/ SP/ W	16S: 1.2–2.5/tyr/D, P1, P1R/ SP/ W	16S: 1.6–2.5/tyr/D, P2R/ SP/ W	16S: 2.0–3.1/tyr/P2 R/ SP/ W	16S: 4.4–5.2/tyr/P2 R/ SP/ W	16S: 3.3–4.3/tyr/P1 N, P2R/ SP/ W	16S: 2.7–3.1/tyr/D, P1, P1R/ SP/ W	16S: 13.4–14.2/tyr/D, P1, P1R/ SP/ W	16S: 11.8–12.0/tyr/D, P1, P1R/ SP/ W				
Ni	16S: 2.7–3.3/tyr/D, P1, P1R/ SP//	16S: 1.9–2.5/tyr/D, P1R/ SP/ W	16S: 4.0–4.4/tyr/D, P1/ SP/ W	16S: 1.8–3.1/tyr/D, P1R/ SP/ W	16S: 2.1–2.7/tyr/D, P1, P1R/ SP/ W	16S: 2.5–4.1/tyr/D, P1, P1R/ SP/ W	16S: 4.5–4.8/tyr/P1, P1R/ SP/ W	16S: 3.9–5.2/tyr/D, P1, P1N/ SP/ W	16S: 3.0–3.5/tyr/P1 R/ SP/ W	16S: 12.0–14.2/tyr/D, P1R/ SP/ W	16S: 11.2–12.0/tyr/D, P1R/ SP/ W	16S: 1.5–2.3/tyr/P1, P1R/ SP/ W			
Po	16S: 2.9–3.1/tyr/D, P1R, P2R// W	16S: 2.1–2.9/tyr/D, P1, P1R/ SP/ W	16S: 5.6/tyr/D, P2N/ SP/ W	16S: 2.7–3.5/tyr/D, P1, P1R/ SP/ W	16S: 2.3–2.7/tyr/P1 R, P2R/ SP/ W	16S: 3.0–4.1/tyr/P1 N// W	16S: 4.4–4.6/tyr/D, P1R// W	16S: 4.8–5.0/tyr/D, P2R/ SP/ W	16S: 3.3/tyr/D, P1, P1R// W	16S: 14.0-14.6/tyr/D, P1, P1R/ SP/ W	16S: 12.4/tyr/D, P1, P1R// W	16S: 2.5–3.1/tyr// SP/ W	16S: 2.7–3.1/tyr/P1, P1N/ SP/ W		
Rw	16S: 2.7–3.3/tyr/D, P2R/ SP/ W	16S: 1.6–2.1/tyr/D, P1, P1R//	16S: 4.8/tyr/D,P 1R, P2N/ SP/ W	16S: 1.8–2.7/tyr/D, P1, P1R/ SP//	16S: 2.3–2.7/tyr/P2 R/ SP/ W	16S: 2.7–3.7/tyr/P1 R// W	16S: 4.1–4.3/tyr/P1 R, P2R/ SP/ W	16S: 4.1–4.5/tyr/P1 N, P2N/ SP/ W	16S: 2.5/tyr/D, P1, P1R/ SP//	16S: 13.4–14.0/tyr/D, P1, P1R/ SP/ W	16S: 10.8/tyr/D, P1, P1R/ SP/ W	16S: 1.9–2.3/tyr/P2 R// W	16S: 1.9–2.3/tyr/P1, P1R/ SP/ W	16S: 2.7/tyr/P1 R/ SP/ W	
Vi	16S: 6.8–7.2/tyr/D, P1R, P2R// SP/ W	16S: 6.6–7.0/tyr/D, P1/ W	16S: 7.7/tyr/D, P1R, P2R/ SP/ W	16S: 5.8–6.8/tyr/D, P1R/ SP/ W	16S: 6.4–6.8/tyr/D, P2R/ SP/ W	16S: 7.0–8.0/tyr/D, P1R, P2R/ SP/ W	16S: 8.5–8.7/tyr/D, P1R. P2R/ SP//	16S: 6.7–7.0/tyr/D, P1R, P2R/ SP/ W	16S: 6.8/tyr/D, P1R/ SP/ W	16S: 14.4-15.1/tyr/D, P1, P1R/ SP/ W	16S: 11.4/tyr/D, P1, P1R/ SP/ W	16S: 6.6–7.1/tyr/D,P 1R, P2R/ SP/ W	16S: 6.8–7.4/tyr/D, P1, P1R/ SP/ W	16S: 7.2/tyr/D, P1R, P2R/ SP/ W	16S: 6.6/tyr/D, P1R, P2R// W

set of 87 specimens using both 12S and 16S sequences. Table 17 shows the minimum and maximum uncorrected p distance values for 16S and summarises all the differences between the species pairs.

A real difficulty faced us in determining the species names that should be applied to the recognised clades (discussed below). The original descriptions are largely undiagnostic, but we adopted the pragmatic approach to best link existing names to the clades we recognize as species. Frost (2011) presents details of older synonymies, which are not repeated here. We assign the clades within the *H. nasutus* group to the following 16 species: *H. acuticeps*, *H. adspersus*, *H. benguellensis*, *H. friedemanni* sp. nov., *H. howelli* sp. nov., *H. igbettensis*, *H. inyangae* sp. nov., *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., *H. nasutus*, *H. nasicus*, *H. poweri*, *H. rwandae* sp. nov., *H. dartevellei* and *H. viridis*. *Hyperolius lamottei* makes up the 16th species.

The phylogenetic analysis including the nuclear gene in addition to the two mitochondrial genes using Bayesian Inference showed an average standard deviation of split frequencies of 0.0032 after 10 million generations. At this point all parameters reported a potential scale reduction factor of 1.000, indicating that the two runs had converged. The tree topology is congruent for both Bayesian Inference and Maximum Likelihood. The Bayesian tree based on the 16S sequences (Figure 28) is displayed with terminal substructure collapsed. Many of the clades show polytomies, which we interpret as evidence of a recent radiation, when read with the uncorrected p distances which vary from 1.4 to 14.4 across the group (Table 17). Although an uncorrected p distance of 3% or more is regarded as indicating a species-level difference (e.g. Fourquet *et al.* 2007), the present study shows that individuals of many species pairs can fall both below and above the 3% mark, such as *H. acuticeps*/*H. dartevellei* (2.3–3.5); *H. adspersus*/ *H. howelli* (1.6–3.3); *H. nasicus*/*H. poweri* (2.7–3.1). The species pairs with low differences show differences in other characters.

The species were recognised by combinations of unique advertisement calls and mitochondrial haplotypes supported by morphological differences. A haplotype network for the nuclear gene tyr, is shown in Figure 29. The heterozygosity H is 0.6 for the group, with a sample of 40 individuals across the species, with 47 tyr haplotypes. Two haplotypes are shared across the hypothesised species boundaries (one includes *H. rwandae* and *H. howelli*; and the other *H. acuticeps*, *H. friedemanni*, *H. dartevellei* and *H. howelli*).

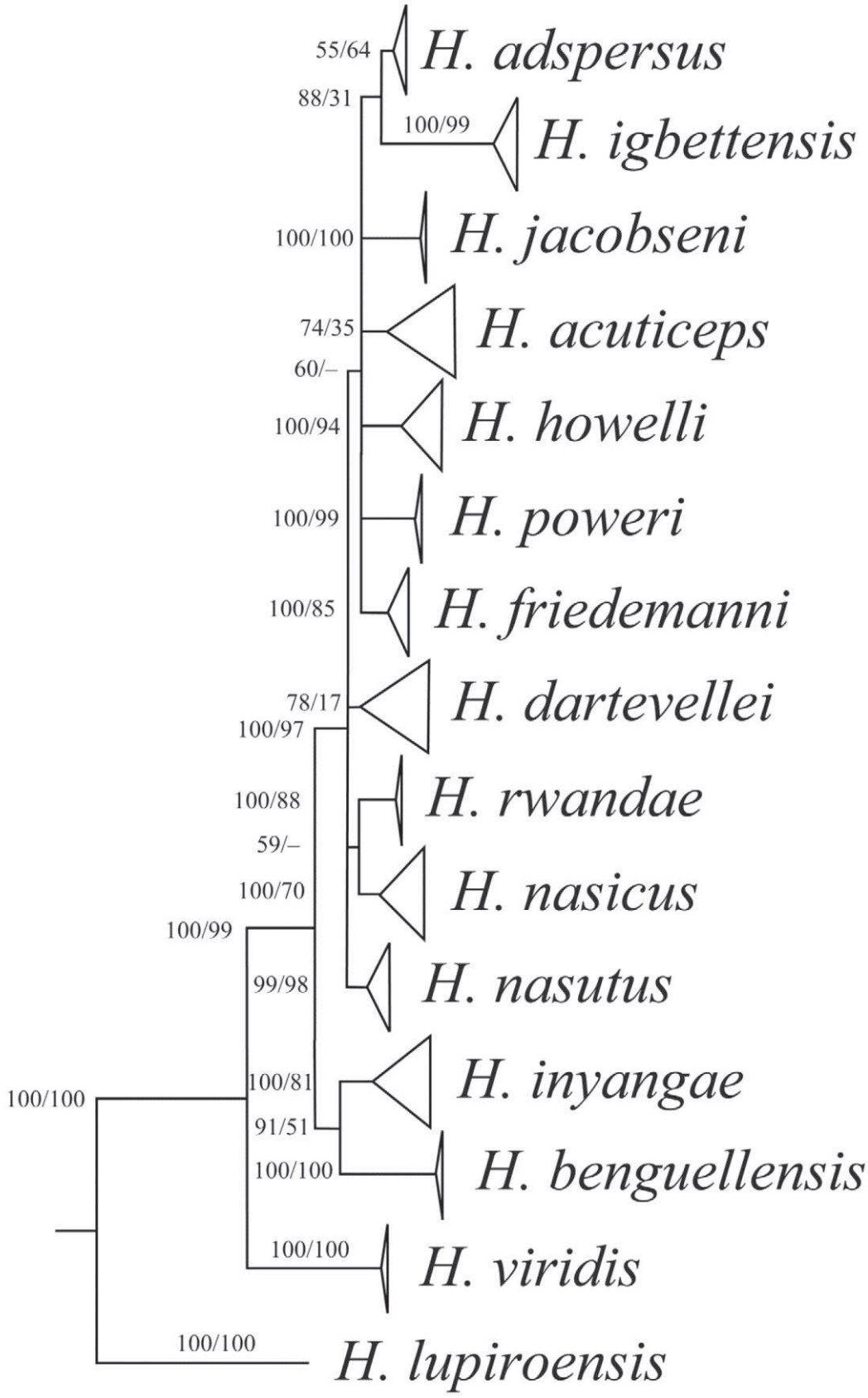

Figure 28. Phylogeny of the recognised species, based on 16S. Tree support is given as posterior probability/ML bootstrap. *Hyperolius lamottei* is basal to the group, and is not shown.

Taxonomy should not be based on sequence differences alone (Moritz & Cicero 2004), although in the *H. nasutus* group there are few morphological characters that can support taxonomic decisions. Although the sample size is too small for any statistical support, there is little inter-species difference in traditional body proportions such as TFL/SUL or HW/SUL. Although these values are not statistically relevant here, they do indicate potentially useful proportions to investigate with larger sample sizes.

The major elements of the dorsal pattern include pale lateral stripes, pale paravertebral stripes, and a dark mid-dorsal line. The pale stripes may have a dark border. The pale stripes are sometimes formed by an absence of dark spots, and sometimes by a very white pigment that remains even after preservation. The dorsum may be speckled or stippled to various degrees. Pale lateral stripes are common in males, with spotted patterns common in females. However, stripes or spots may be found in both sexes. The results of the morphological study are included with the relevant species below, and the measurements are summarised in Appendix 8.

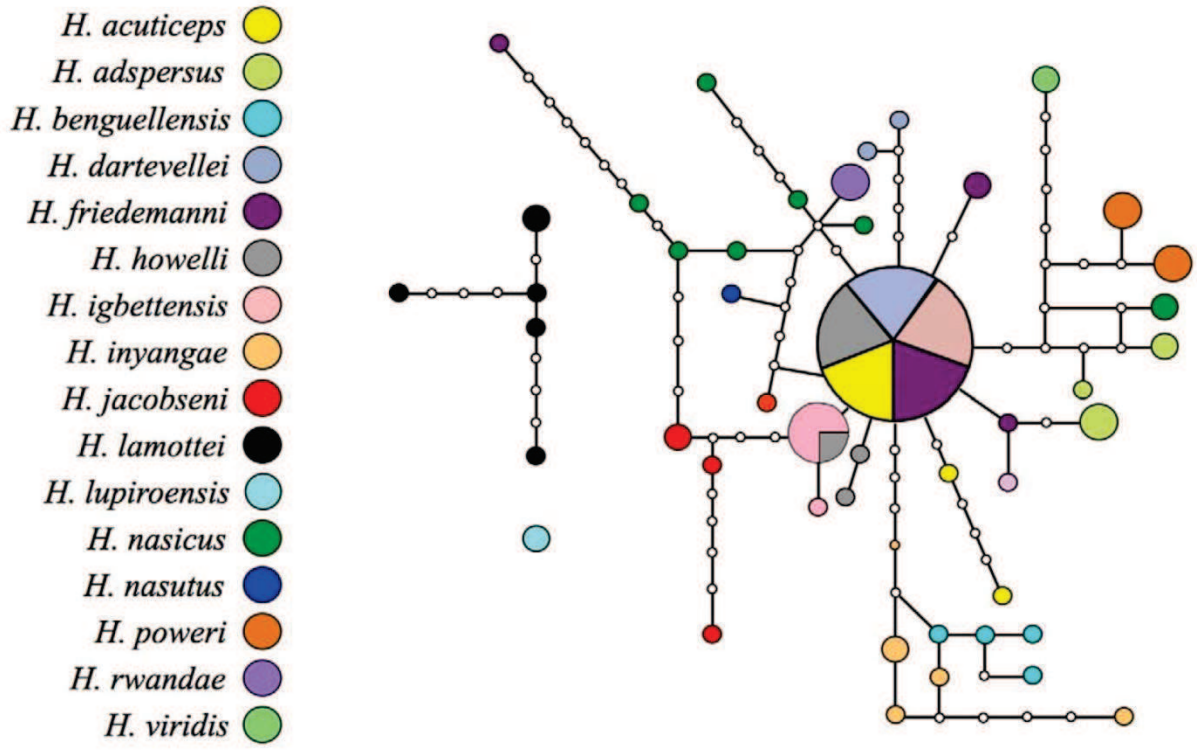

Figure 29. Haplotype network of the nuclear tyr gene. Each circle represents a unique haplotype. Species are indicated in colour, and hypothesised intermediates are shown as solid circles. The size of the circle indicates the number of individuals sharing the haplotype. One haplotype (large circle) is shared by individuals from five species, and a second is shared by two species. *Hyperolius lamottei* and *H. lupiroensis* haplotypes are more than nine changes different from the network.

Table 18. Summary of advertisement calls. For each parameter the mean, (range) and sample size are given.

Species	Emphasised frequency (Hz)	Duration (s)	First phase length (s)	First phase number of pulses	First phase pulse rate (s^{-1})	Second phase length (s)	Second phase number of pulses	Second phase pulse rate ($^{s-1}$)
H. acuticeps	4207 (3949–4443) n=7	0.354 (0.306–0.395) n=7	0.026 (0.025–0.028) n=7	5 (5–5) n=7	193 (179–200) n=7	0.329 (0.279–0.367) n=7	10 (9–11) n=7	30 (29–32) n=7
H. adspersus	4631 (4511–4692) n=3	0.037 (0.036–0.038) n=3	0.037 (0.036–0.038) n=3	15 (13–17) n=3	414 (351–447) n=3			
H. benguellensis	4211 (4148–4363) n=5	0.399 (0.372–0.422) n=5	0.038 (0.034–0.041) n=5	5.6 (5–7) n=5	147 (121–179) n=5	0.37 (0.336–0.420) n=5	12.8 (12–14) n=5	34 (33–35) n=5
H. dartevellei	4752 (4556–4907) n=4	0.052 (0.045–0.056) n=4	0.052 (0.045–0.056) n=4	19.5 (19–20) n=4	373 (351–422) n=4			
H. friedemanni **sp. nov.**	4497 (4381–4545) n=8	0.143 (0.104–0.156) n=8	0.042 (0.037–0.050) n=8	8.5 (7–10) n=8	202 (166–219) n=8	0.103 (0.064–0.116) n=8	6.3 (4–7) n=8	60 (57–62) n=8
H. howelli **sp. nov.**	4271 (4187–4331) n=6	0.161 (0.132–0.193) n=6	0.06 (0.05–0.07) n=6	9 (8–10) n=6	151 (142–160) n=6	0.102 (0.079–0.142) n=6	3.7 (3–5) n=6	36 (34–37) n=6
H. igbettensis	4467 (4348–4616) n=7	0.142 (0.088–0.198) n=7	0.051 (0.039–0.063) n=7	12.4 (10–15) n=7	245 (233–260) n=7	0.092 (0.050–0.155) n=7	5.1 (3–8) n=7	57 (51–64) n=7
H. inyangae	4064 (3846–4385) n=8	0.295 (0.177–0.332) n=8	0.026 (0.021–0.031) n=8	4.6 (4–6) n=8	138 (129–238) n=8	0.271 (0.168–0.309) n=8	8.8 (6–10) n=8	32 (29–35) n=8
H. jacobseni **sp. nov.**	3640 (3538–3702) n=7	0.078 (0.067–0.089) n=7	0.078 (0.067–0.089) n=7	5.7 (5–7) n=7	73 (69–79) n=7			
H. lamottei	3545 (3500–3679) n=6	0.073 (0.068–0.077) n=6	0.073 (0.068–0.077) n=6	28.3 (28–29) n=6	388 (376–426) n=6			
H. lupiroensis **sp. nov.**	3693 (3529–3823) n=8	0.058 (0.040–0.072) n=8	0.058 (0.040–0.072) n=8	23.3 (17–30) n=8	401 (362–450) n=8			
H. nasicus	3508 (3430–3535) n=7	0.121 (0.086–0.217) n=7	0.121 (0.086–0.217) n=7	13.7 (10–22) n=7	116 (101–135) n=7			
H. nasutus	4484 (4213–4600) n=4	0.159 (0.145–0.179) n=4	0.054 (0.053–0.055) n=4	10 (8–13) n=4	186 (151–236) n=4	0.104 (0.086–0.124) n=4	5 (4–6) n=4	48 (46–49 n=4
H. poweri	4558 (4389–486[illegible]) n=6	0.137 (0.113–0.150) n=6	0.048 (0.039–0.071) n=6	6.7 (5–9) n=6	139 (113–159) n=6	0.088 (0.068–0.110) n=6	3.8 (3–5) n=6	43 (36–52) n=6
H. rwandae **sp. nov.**	476[illegible] (4728–484[illegible]) n=7	0.137 (0.108–0.183) n=7	0.058 (0.050–0.071) n=7	12 (10–15) n=7	205 (181–232) n=7	0.079 (0.049–0.112) n=7	3 (2–4) n=7	38 (34–41) n=7
H. viridis	350[illegible]	0.473	0.131	11	84	0.347	5	14.4

Taxonomy

Hyperolius acuticeps Ahl, 1931

Sharp-headed Long Reed Frog (Figure 30)

Genetic material: MCZ A-137085–86 (Chelinda Camp, Nyika Plateau, Malawi); ZMB 76103, 76107, 76109 (Chongoni Forest Reserve, Malawi); ZMB 76097–98 (Kaningina Forest Reserve, Malawi) (Figure 27).

Diagnosis: The illustrated advertisement call (Figure 31) has a duration of 0.22 s, consisting of 25 pulses, with a slower pulse rate at the end. This differs from the brief single notes of *H. adspersus*, *H. lupiroensis* sp. nov., and the brief note consisting of a few initial pulses, followed by a number of pulses at a much slower pulse rate, such as *H. benguellensis*, *H. friedemanni* sp. nov., *H. howelli* sp. nov., *H. igbettensis*, *H. inyangae* sp. nov., *H. rwandae* sp. nov., *H. viridis*, and *H. poweri*. The structure of the call of *H. jacobseni* sp. nov. and *H. nasutus* is similar to that of *H. acuticeps*, but the former consists of only five pulses with a duration of 0.07 s, while the latter consists of eight pulses in 0.1 s. The call of *H. dartevellei* consists of 13 pulses in 0.1 s. See Table 18 for a summary of call parameters. The snout is sharply rounded in profile and from above, differing from the truncated snout of *H. dartevellei* and the sharp, shark-like profile of *H. benguellensis*, *H. inyangae* sp. nov. or the bluntly rounded snout profile of *H. adspersus*, *H. igbettensis*, *H. jacobseni* sp. nov., and *H. poweri*. Although the webbing is variable, a typical specimen has one phalanx of the fifth toe free, while the fourth toe is webbed with half to just more than the first phalanx free and the third toe likewise. It can be distinguished from species with less than one phalanx of the fifth toe free, such as *H. adspersus*, *H. friedemanni* sp. nov., *H. igbettensis*, *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., *H. poweri*, *H. rwandae* sp. nov. and *H. viridis*. It differs from those species with more than one phalanx free; *H. howelli* sp. nov. and *H. inyangae* sp. nov. It differs from *H. benguellensis* which has webbing on the third toe extending to the disc, from *H. nasutus* which has webbing on the fourth toe reaching the disc, and from *H. dartevellei* which has half a phalanx of the fourth toe free.

Description of a Chelinda specimen: This description is based on a female MCZ A-137085 from Chelinda on Nyika Plateau. Body long and slender, widest at temporal region, slightly tapering to groin; head comparatively small (HL/SUL 0.33, HW/SUL 0.30), not wider than

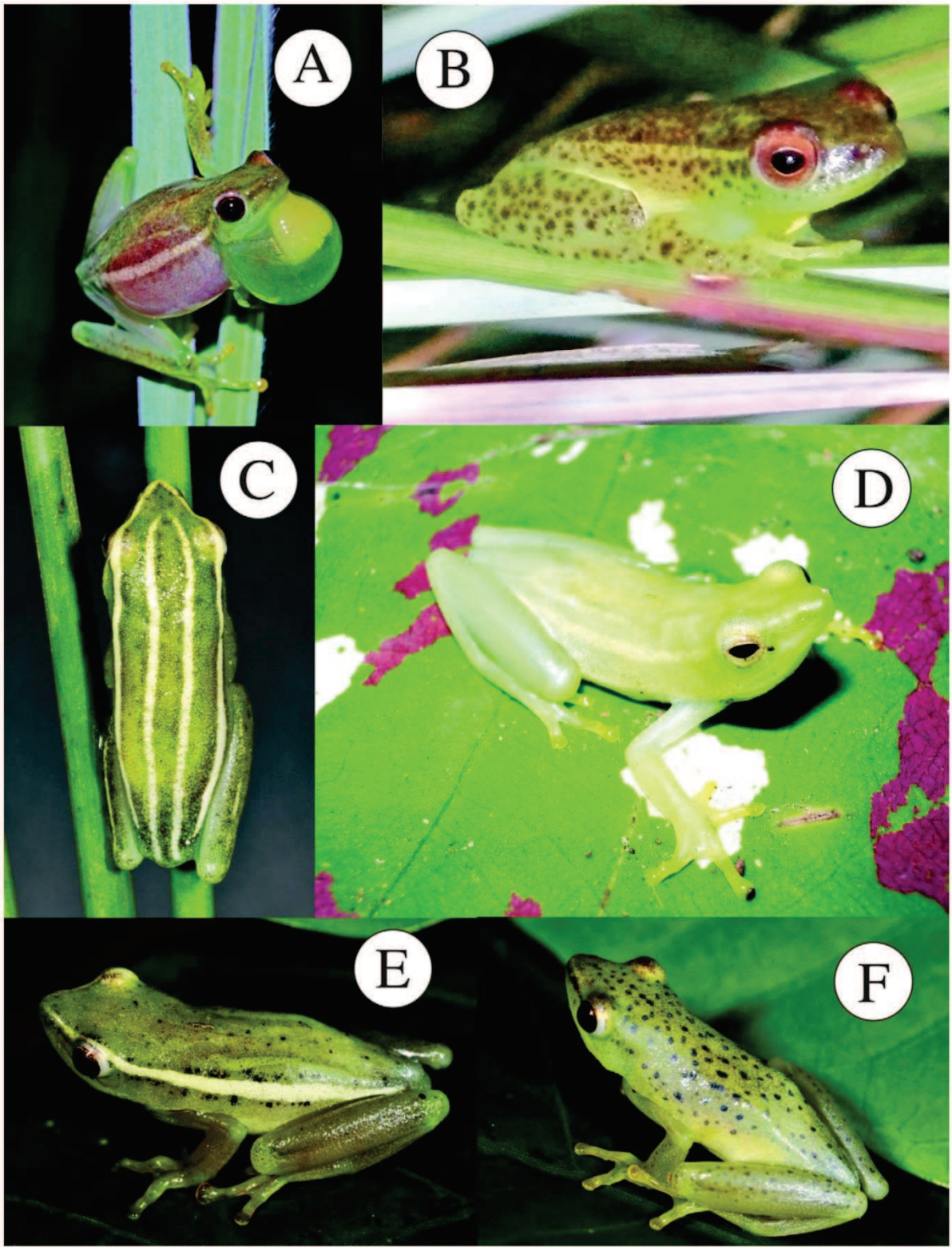

Figure 30. (A) *Hyperolius acuticeps*, Chongoni, Malawi, photo V. Mercurio; (B) *H. adspersus*, Bateka Nature Reserve, Gabon, photo G. Jongsma; (C) *H. benguellensis*, Humpata, Angola, photo A. Channing; (D) *H. friedemanni* sp. nov., Karonga, Malawi, photo V. Mercurio; (E) *H. howelli* sp. nov., holotype, SAIAB 118979, Arusha, Tanzania, photo A. Channing; (F) *H. howelli* sp. nov., female paratype, SAIAB 118980-1, Arusha, Tanzania, photo A. Channing.

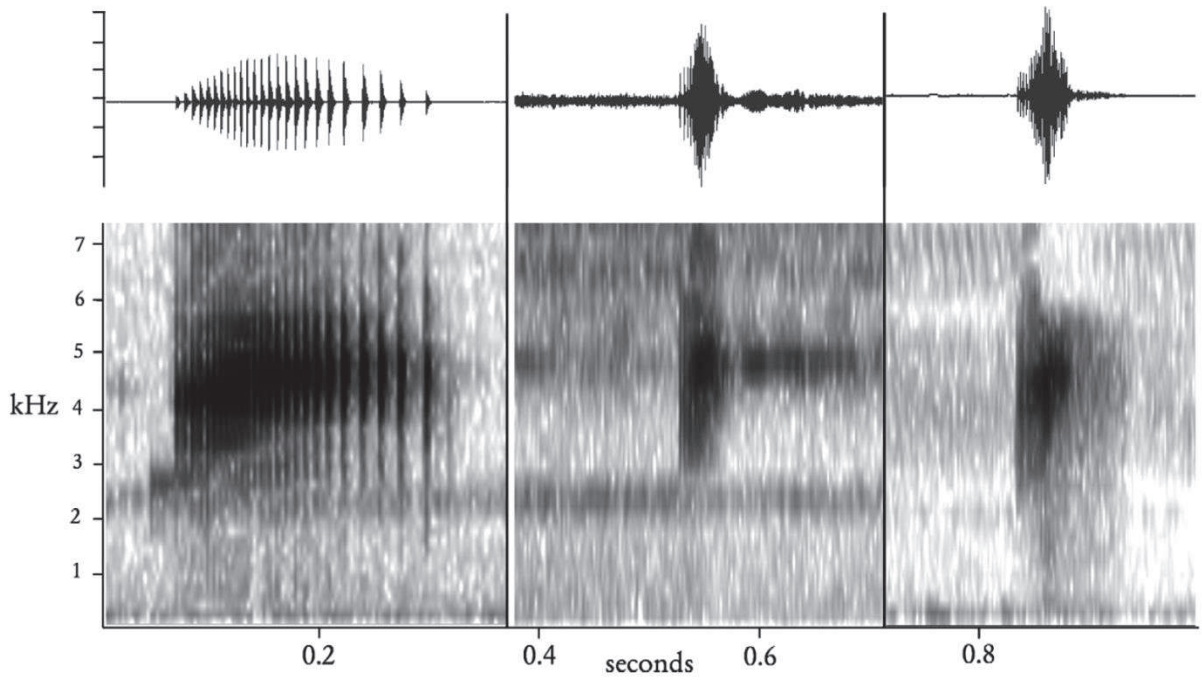

Figure 31. Advertisement calls of *H. acuticeps*, Kaningina (left); *H. adspersus*, Bateka Nature Reserve (center); and *H. dartevellei*, Carumbo (right).

trunk, longer than wide (HL/HW 1.10) although slightly wider than long in specimen MCZ A-137086; snout long (SL/HL 0.43), pointed in dorsal view, acute in profile (Figure 32), considerably projecting beyond lower jaw, wider than long (SL/EE 0.72); canthus rostralis distinct, rounded, slightly concave from eye to nostril, concave near tip of snout; loreal region almost vertical, slightly concave; nostril directed laterally; situated much closer to tip of snout than to eye (EN/NS 1.43), separated from each other by distance less than distance between eye and nostril (NN/EN 0.9); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.26); eye diameter shorter than snout (ED/SL 0.61); interorbital distance as wide as upper eyelid (IO/EW 1.0), and greater than internarial distance (IO/NN 1.67); tympanum not visible externally; upper jaw with dentition; teeth on premaxilla larger than those on maxilla; choanae small, oval, located far anterolaterally at margins of roof of the mouth; vomer processes and teeth absent; tongue long (4.8), and wide (3.7 at widest point), free for about three-fourths of length, bifurcated distally for about one-fourth of length; median lingual process absent.

Dorsal surfaces of head, trunk and limbs smooth; ventral surface of limbs and gular smooth, chin and abdomen slightly more areolate.

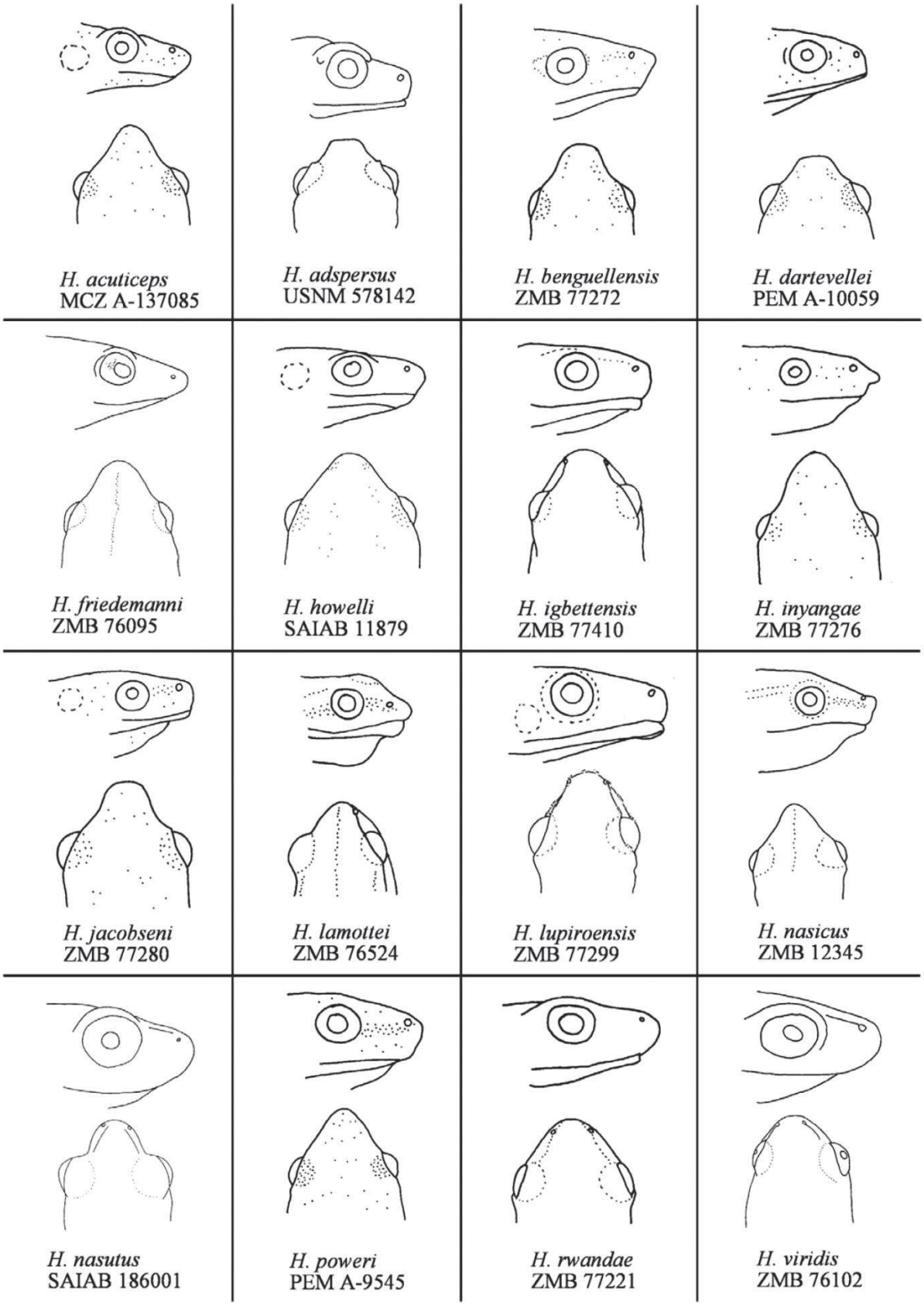

Figure 32. Snout profiles of representatives of the species in the *Hyperolius nasutus* group.

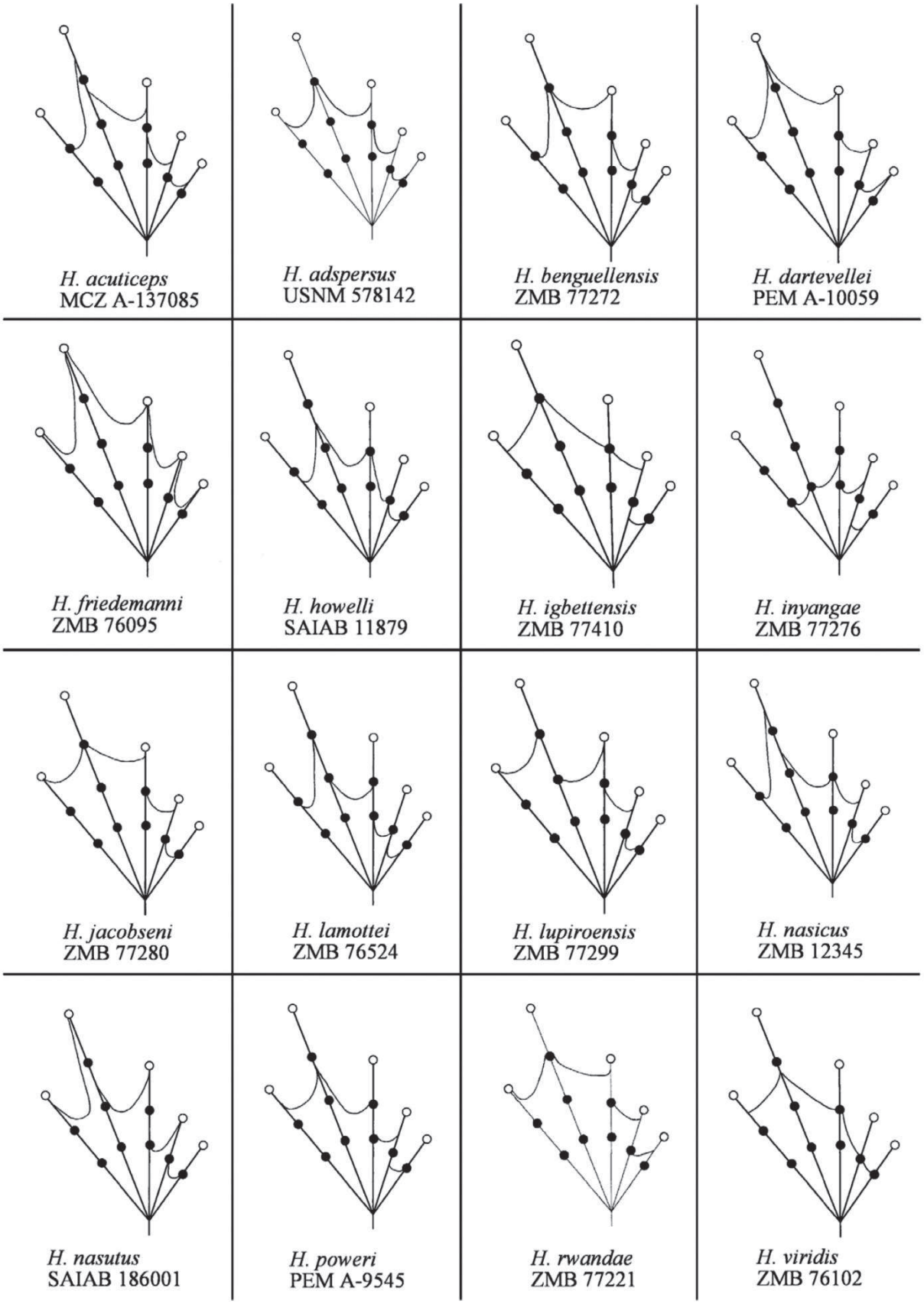

Figure 33. Webbing diagrams of representatives of the species in the *Hyperolius nasutus* group.

Forelimbs slender; hand moderately large (HND/SUL 0.27); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV, with proximal tubercle on finger IV hardly discernible; webbing formula of the hand I1–2II1–2.5III2–1IV (after Myers & Duellman [1982]); thenar tubercle indistinct, low; palmar tubercles absent; inner metacarpal tubercle small, rounded, outer metacarpal tubercle absent.

Hindlimbs slender, moderately long (LEG/SUL 1.45); tibio-tarsal articulation reaching to level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.49), longer than thigh (TFL/THL 1.07); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.82); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1.5–2II1–2III1–2IV2–1.5V; inner metatarsal tubercle small, oval, prominent; outer one larger, almost circular, low and almost indistinct. Measurements of a second specimen from Chelinda MCZ A-137086 are included in Appendix 8.

Colouration in preservative: The pattern elements that remain after the green has faded are a dark vertebral stripe made up of a single row of chromatophores, with pale lateral bands bordered by irregular dark spots, and a row of dark chromatophores forming a dorsolateral stripe. A male is illustrated in Figure 30.

Eggs and tadpoles: Unknown.

Distribution: This species is presently only confirmed from northern Malawi on the Nyika Plateau and Chongoni and Kaningina Forest Reserves, and the type locality in southern Tanzania.

Remarks: This study restricts the distribution of the species from the wide range presently attributed to it (Schiøtz *et al.* 2004), suggesting that its conservation status should be changed from Least Concern to Data Deficient, pending the collection of more data.

Hyperolius adspersus Peters, 1877

Sprinkled Long Reed Frog (Figure 30)

Synonymy: *Hyperolius granulatus* (Boulenger, 1901)

Genetic material: USNM 578140, 578144, 578166 (Plain of Vera, 15 km south east of Gamba, Gabon); USNM 578157 (Uemba Road, 2 km south of Gamba, Gabon); USNM 578142 (Setecama Road, 3 km west of Gamba, Gabon); USNM 578165 (National Forestry School, Gabon) (Figure 27). Specimens examined as above, including the type (ZMB 917).

Diagnosis: The advertisement call consists of a brief note, duration 0.04 s, and indistinguishable pulses (Figure 31). It can be distinguished from the call consisting of a brief note comprising a few initial pulses, followed by a number of pulses at a much slower pulse rate, such as *H. benguellensis*, *H. friedemanni* sp. nov., *H. howelli* sp. nov., *H. igbettensis*, *H. inyangae* sp. nov., *H. rwandae* sp. nov., *H. viridis*, and *H. poweri*. It differs from the longer calls consisting of a number of pulses at a more or less constant rate, such as *H. acuticeps*, *H. jacobseni* sp. nov., *H. nasutus*, *H. nasicus*, and *H. dartevellei*. See Table 18 for a summary of call parameters. The snout is truncated to bluntly rounded, which differs from the sharp, shark-like profile of *H. benguellensis* and *H. inyangae* sp. nov., and the sharply rounded snout profile of *H. acuticeps*, *H. friedemanni* sp. nov., *H. lupiroensis* sp. nov., *H. nasutus*, and *H. rwandae* sp. nov. The fifth toe has about half a phalanx free of web. This distinguishes it from those species that have one or more phalanges of the fifth toe free, *H. benguellensis*, *H. howelli*, *H. inyangae*, *H. lamottei*, and *H. nasicus*; and those with the fifth toe fully webbed, *H. friedemanni*, *H. jacobseni*, *H. lupiroensis*, and *H. rwandae*. It has one phalanx free of webbing on the fourth toe, which distinguishes it from *H. dartevellei*, which has less than a phalanx free; *H. nasutus* which is webbed to the disc at least on one side; and *H. poweri* and *H. viridis* which have more than one phalanx free. It has no more than one phalanx free on the second toe, at least on one side, which distinguishes it from *H. igbettensis* which has more than one phalanx free of the second toe at least on one side.

Description of a specimen from Gamba, Gabon: An adult male USNM 578142 (measurements presented in Appendix 8) measuring 19.5 SUL; body long and slender, widest just behind orbital region, tapering to groin; head relatively small (HL/SUL 0.27, HW/SUL 0.33), wider than long (HL/HW 0.82); snout long (SL/HL 0.51, truncated in dorsal view (Figure

32), just protruding just beyond lower jaw, wider than long (SL/EE 0.64); canthus rostralis rounded; loreal large and oval in shape; nostril directed laterally, elliptical slit, situated just behind the tip of the snout (EN/NS 1.60), separated from each other by distance greater than distance between eye and nostril (NN/EN 1.19); eyes large (ED 2.0), directed anterolaterally, protruding outwards and forward, pupil is horizontal to circular, visible from below, eye diameter shorter than snout (ED/SL 0.74); interorbital distance much wider than upper eyelid (IO/EW 1.46), and equal to internarial distance (IO/NN 1.0); tympanum not visible externally; upper jaw with dentition; teeth on premaxilla larger than those on maxilla; choanae large, oval, vomer processes and teeth absent; tongue long and broad, mostly free except for first quarter, bifurcated distally for about one-fourth of length; median lingual process absent; vocal sac single, median, subgular; large granular gular flap covering thin vocal sac (4.3 wide).

Dorsal surfaces of head, trunk and limbs generally appearing smooth but with many densely and more or less evenly scattered tiny, melanophores; ventral surface of limbs and gular smooth, chin and abdomen slightly more areolate; supratympanic fold absent.

Forelimbs slender; hand moderately large (HND/SUL 0.28); tips of fingers enlarged into broad oval disks, thin circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one per phalange; webbing formula of the hand I2.5–2.5II2–2.25III2.25–2IV; thenar tubercle indistinct; palmar tubercles absent.

Hindlimbs slender, moderately long; tibiofibula moderately long (TFL/SUL 0.54), longer than thigh (TFL/THL 1.08); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.85); relative length of toes: I<II<III<V<IV; discs of toes similar in size to those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1–2II1–2III1–2IV2–1V; inner metatarsal flat; outer metatarsal tubercle absent.

Colouration in life: No information, but see the photo (Figure 30) of an unvouchered individual from the Bateka Nature Reserve, Gabon.

Colouration in preservative: All colours have faded to a beige yellow with evenly spaced black dorsal melanophores.

Eggs and tadpoles: Unknown.

Habitat: Specimens were collected in grassland.

Distribution: Southern Cameroon, east and south through Gabon to the lower Congo Basin.

Remarks: The synonymy of *H. granulatus* (the holotype RMCA-152 was examined) is supported by the absence of dorsolateral stripes and a short rounded snout. The species is presently only confirmed from northern Angola, the Cabinda enclave, and Gabon. There is little doubt that existing records refer to this species, and we suggest that its conservation status of Least Concern remains unchanged.

Hyperolius benguellensis (Bocage, 1893)
Benguella Long Reed Frog (Figure 30)

Synonymy: *Hyperolius oxyrhynchus* (Boulenger, 1901)

Genetic material: ZMB 77271–2, ZMB 77318 (Humpata, Angola); ZMB 77273–4 (Bicuar National Park, Angola); ZMB 77275 (Zootecnica Plateau, Humpata, Angola); AACRG 1030 (Kaparotta, Botswana); GenBank AF215224, AF215442 (Rundu, Namibia) (Figure 27).

Diagnosis: The advertisement call (Figure 34) is a brief note consisting of five pulses, followed by 14 pulses at a slower rate, with a duration of 0.41 s. It can be distinguished from the brief calls consisting only of a single note, *H. adspersus* and *H. lupiroensis* sp. nov. and those consisting only of a series of pulses, *H. acuticeps*, *H. jacobseni* sp. nov., *H. nasutus*, and *H. dartevellei*. The other species with advertisement calls consisting of an initial note followed by some discrete pulses can be distinguished either by their short duration, less than 0.2 s, as in *H. friedemanni* sp. nov., *H. howelli* sp. nov., *H. igbettensis*, *H. poweri*, and *H. rwandae* sp. nov., or by the lower number of slow pulses, less than 10, as in *H. inyangae* and *H. viridis*. See Table 18 for a summary of call parameters. The snout is shark-like in profile, protruding forward of the mouth in a straight line, before forming a sharp tip. It can be distinguished from the truncated, sharply- or bluntly rounded snout profiles as in *H. acuticeps*, *H. adspersus*, *H. friedemanni* sp. nov., *H. igbettensis*, *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., *H. nasutus*, *H. poweri*, *H. rwandae* sp. nov., and *H. viridis*.

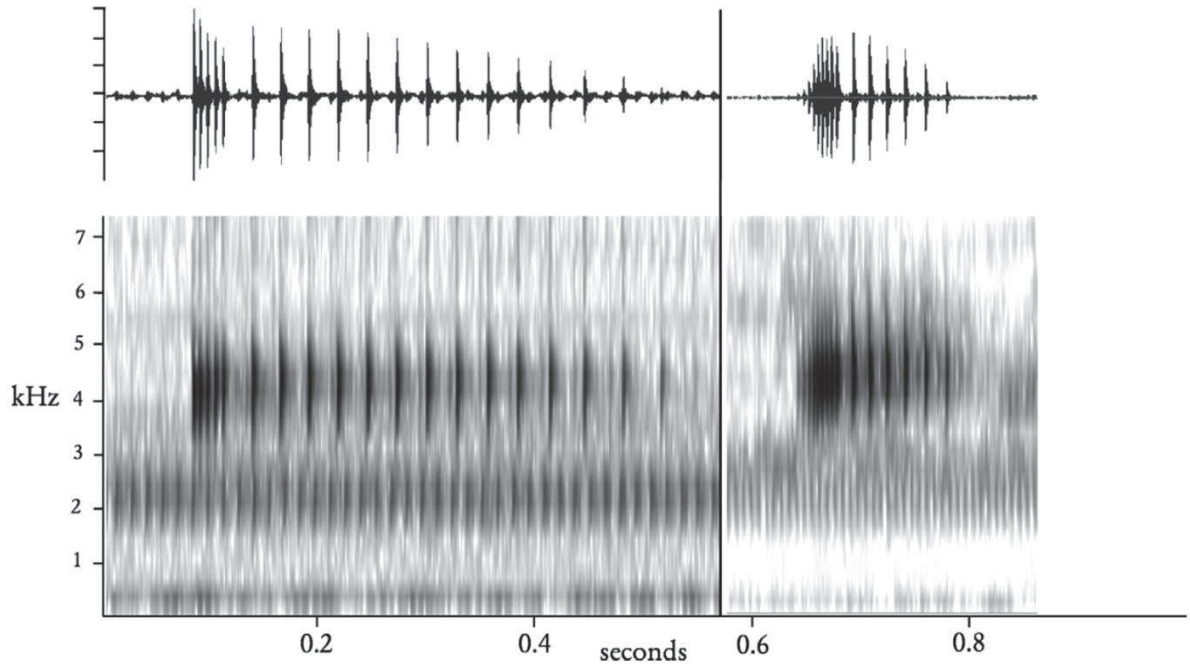

Figure 34. Advertisement calls of *H. benguellensis*, Humpata (left) and *H. friedemanni*, Karonga (right).

Description of a Humpata specimen: Body long and slender, widest at mid-body, slightly tapering to groin; head comparatively small (HL/SUL 0.33, HW/SUL 0.30), not wider than trunk, longer than wide (HL/HW 1.11); snout long (SL/HL 0.42), bluntly rounded in dorsal view, acute, sharklike in profile (Figure 32), considerably projecting beyond lower jaw, wider than long (SL/EE 0.68); canthus rostralis distinct, moderately sharp, slightly concave from eye to just beyond nostril, slightly convex near tip of snout; loreal region almost vertical, slightly concave; nostril directed dorsolaterally; situated much closer to tip of snout than to eye (EN/NS 1.64), separated from each other by distance greater than distance between eye and nostril (NN/EN 1.11); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.33); eye diameter shorter than snout (ED/SL 0.78); interorbital distance much wider than upper eyelid (IO/EW 1.71), and greater than internarial distance (IO/NN 2.1); tympanum not visible externally; upper jaw with dentition; teeth on premaxilla larger than those on maxilla; choanae small, oval, located far anterolaterally at margins of roof of the mouth, vomer processes and teeth absent; tongue long (5.8), and narrow (2.7 at widest point), free for about three-fourths of length, bifurcated distally for about one-third of length; median lingual process absent; vocal sac single, median, subgular, mostly unpigmented and translucent when fully inflated; gular flap cream-coloured, granular; vocal

sac aperture on each side of the mouth, situated lateral from and close to base of tongue, slit-like, long, directed posterolaterally.

Dorsal surfaces of head, trunk and limbs smooth; ventral surface of limbs and gular smooth, chin and abdomen slightly more areolate.

Forelimbs slender; hand moderately large (HND/SUL 0.30); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV, with proximal tubercle on finger IV hardly discernible; webbing formula of the hand I2–2.5II2–3III2.5–2.5IV (after Myers & Duellman [1982]); thenar tubercle absent; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hindlimbs slender, moderately long (LEG/SUL 1.55); tibio-tarsal articulation reaching to level of snout tip when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.53), shorter than thigh (TFL/THL 0.91); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.83); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1–1II0.75–1III0–1IV1–1V; inner metatarsal tubercle small, oval, prominent; outer one low, almost indistinct.

Colouration in life: Skin more or less translucent. Dorsum and dorsal surface of head and limbs dark green (Figure 30); lateral sides of head and scapular region dark green; light, yellowish-white, moderately broad dorsolateral stripe running along each side of the body from snout tip, over the eye to vent; Pale paravertebral stripes originating on posterior of snout, diverging to level of eyes, and then running parallel to vent; small dark brown to black dots and on dorsum, most densely bordering both sides of dorsolateral stripes; distal portions of fingers and toes, especially the tips, yellow; ventral side and parts of dorsal side of thigh and upper arm largely unpigmented but with irregular dark spots, appearing bluish-green; peritoneum white, shining through the translucent belly skin. Iris reddish-brown.

Colouration in preservative: All colours have faded to yellow; gular flap whitish-yellow.

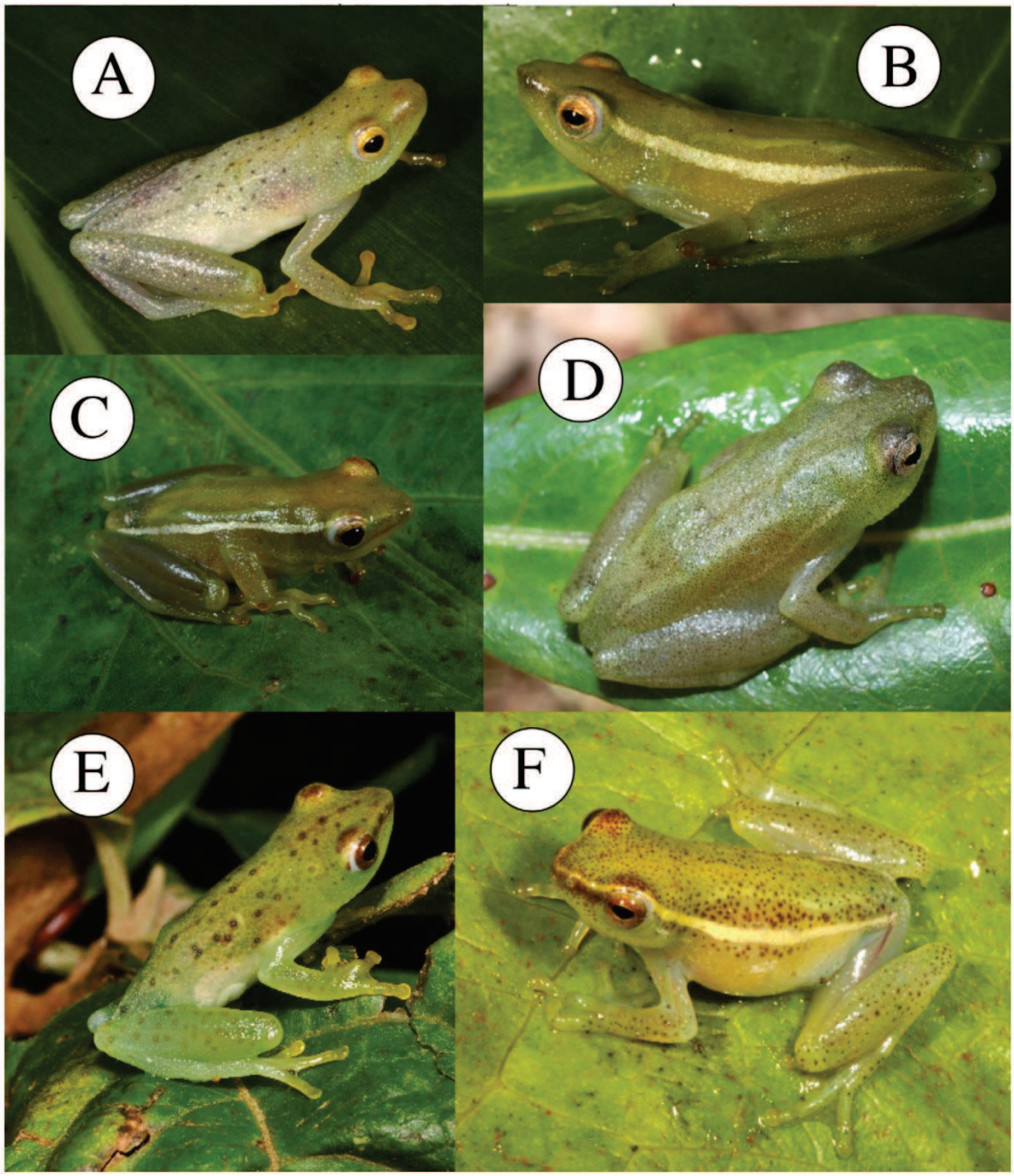

Figure 35. (A) *Hyperolius nasutus*, Calandula, Angola, ZMB 77311, photo A. Channing; (B) *Hyperolius poweri*, Mkambati NR, South Africa, photo W. Conradie; (C) *Hyperolius rwandae* sp. nov., Butare, Rwanda, ZMB 77221, holotype, photo J.M. Dehling; (D) *Hyperolius viridis*, Kaningina, Malawi, photo V. Mercurio; (E) *Hyperolius rwandae* sp. nov, female paratype ZMB 77223, photo J.M Dehling; (F) *Hyperolius dartevellei*, Carumbo, Angola, PEM A 10035, photo W. Conradie.

Variation: The female ZMB 77271 is similar to the male in measurements (Appendix 8). The female is larger than the male (SUL 23.5). Colouration is variable; ZMB 77271 has a pale green dorsum with irregular darker marks, and yellow eyelids, with fingers and toes also

yellow, and a white underside. AC 3073 has pale dorsolateral stripes on a dark green background.

Eggs and tadpoles: Unknown.

Habitat: We found the species only in open grassy habitats, along stream banks and man-made impoundments where sedges and other tall emergent vegetation were present (Humpata, Bicuar NP). Specimens were observed on leaves and stems of vegetation between 5 cm and 1.0 m above water level. Males called from elevated positions. The following species were found sympatrically or even syntopically with the new species*: Hyperolius marmoratus* and *Xenopus laevis*.

Remarks: The species is confirmed using molecular data from Rundu in the Caprivi Strip of Namibia, northern Botswana, and southern Angola. The type locality of *H. benguellensis* is Caota, Angola. The type series (MBL 17.220-223; now Museu Bocage, National Museum of Natural History, University of Lisbon) has been destroyed (Frost 2011). Our specimens agree with the original description of a sharp snout and small dorsal speckles. The specimens from Humpata show a range of colour patterns, from a uniform finely spotted dorsum, to pale dorsolateral stripes, to dorsolateral and paravertebral stripes. This variation was absent from the type description. The genetic material is from the same drainage basin as the type. *Hyperolius oxyrhynchus* is regarded as a synonym as the type description matches this species. The species is presently only confirmed from southern Angola, northern Namibia, and northern Botswana. We suggest that the conservation status Least Concern be maintained.

Hyperolius dartevellei Laurent, 1943
Dartevelle's Reed Frog (Figure 35)

Synonymy: *Hyperolius sagitta* Laurent, 1943

Genetic material: ZMB 77303 Ikelenge, Zambia; USNM 576167–70 (Impongui, Republic of Congo); field numbers A27, CRT 3577-9, 3604-6 (Congo River near Yekela, DRC); CRT 3730, 3798 (Congo River, near Nganda Kona, DRC); CRT 3838-9 (Congo River near Ngengele, DRC); CRT 3975–89 (Congo River near Bomani, DRC); CRT 4024, 4027 (Congo River, near Lulu,

DRC); CRT 4205–10 (Congo River, near Lieki, DRC) (Figure 27).

Diagnosis: A typical advertisement call (Figure 31) consists of 13 pulses in 0.1 s, with an emphasised frequency of 4.8 kHz. It differs from those species with a brief note consisting of a few initial pulses, followed by a number of pulses at a much slower pulse rate, such as *H. benguellensis*, *H. friedemanni* sp. nov., *H. howelli* sp. nov., *H. igbettensis*, *H. inyangae* sp. nov., *H. rwandae* sp. nov., *H. viridis*, and *H. poweri*, and those with a longer call consisting of multiple pulses that may change tempo, such as *H. acuticeps*, *H. jacobseni* sp. nov., and *H. nasutus*. See Table 18 for a summary of call parameters. The advertisement call structure is similar to that of *H. adspersus* and *H. lupiroensis* sp. nov., while the 16S sequence of *H. lupiroensis* sp. nov. differs by more than 11%. The snout is truncated, distinguishing it from the species with shark-like or rounded snout profiles: *H. acuticeps*, *H. adspersus*, *H. benguellensis*, *H. friedemanni* sp. nov., *H. howelli* sp. nov., *H. igbettensis*, *H. inyangae* sp. nov., *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., *H. nasutus*, *H. poweri*, *H. rwandae* sp. nov., and *H. viridis*. The webbing shows a phalanx free on the first, third and fifth toes, with half a phalanx free on the other two. It can be distinguished from the species that have less than a phalanx of the fifth toe free: *H. adspersus*, *H. friedemanni* sp. nov., *H. igbettensis*, *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., *H. nasutus*, *H. poweri*, *H. rwandae* sp. nov., and *H. viridis*. It differs from the species that have one phalanx or more of the fourth toe free of web: *H. acuticeps*, *H. benguellensis*, *H. howelli* sp. nov., and *H. inyangae* sp. nov.

Description of a Carumbo specimen: An adult male PEM A 10059 (measurements presented in Appendix 8) measuring 18.6 SUL; body long and slender, widest just behind orbital region, tapering to groin; head relatively small (HL/SUL 0.32, HW/SUL 0.34), not much wider than long (HL/HW 0.95); snout long (SL/HL 0.46), bluntly pointed in dorsal view (Figure 32), protruding just beyond lower jaw, wider than long (SL/EE 0.72); canthus rostralis distinct; loreal large and oval in shape; nostril directed dorsolaterally, moderately large vertical slit (0.4 in length), situated much closer to tip of snout than to eye (EN/NS 1.60), separated from each other by distance greater than distance between eye and nostril (NN/EN 1.60); eyes large (ED 1.8), directed anterolaterally, protruding outwards and forward, pupil is horizontal to circular, visible from below, eye diameter shorter than snout (ED/SL 0.64); interorbital distance much wider than upper eyelid (IO/EW 1.50), and greater than internarial distance (IO/NN 1.41); tympanum not visible externally; upper jaw with dentition; teeth on premaxilla

larger than those on maxilla; choanae large, oval, vomer processes and teeth absent; tongue long (3.9) and broad (2.8), mostly free except for first quarter, bifurcated distally for about one-fourth of length; median lingual process absent; vocal sac single, median, subgular; large granular gular flap covering thin vocal sac (5.9 wide).

Dorsal surfaces of head, trunk and limbs generally appearing smooth but with many densely and more or less evenly scattered tiny, asperities; ventral surface of limbs and gular smooth, chin and abdomen slightly more areolate; supratympanic fold absent.

Forelimbs slender; hand moderately large (HND/SUL 0.26); tips of fingers enlarged into broad oval disks, no circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one per phalange; webbing formula of the hand I1.5–0.25II0.25–0.25III0.25–0.25IV (after Myers & Duellman 1982) thenar tubercle indistinct; palmar tubercles absent.

Hindlimbs slender, moderately long (LEG/SUL 1.50); tibio-tarsal articulation reaching to level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.52), longer than thigh (TFL/THL 1.07); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.77); relative length of toes: I<II<III<V<IV; discs of toes similar in size to those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I0.25–1II0.25–1III0.25–1IV1–0.25V; inner metatarsal absent; outer metatarsal tubercle large, almost circular, low and not distinct.

Colouration in life: Below translucent silvery-white, above uniform translucent green to brown, scattered darker spots, clear yellow-white dorsolateral line from snout to vent, forming a light canthus on the snout, darker pigmentation anterior-lateral from snout tip to above eye, upper jaw nearly free of any pigmentation, iris yellow to brown; dorsal surface of arms and legs with scattered dark spots, inner thighs unmarked.

Colouration in preservative: All colours have faded to a beige yellow with brown dorsal spots still visible.

Eggs and tadpoles: Unknown.

Habitat: Specimens were collected in the grassland floodplain wetlands surrounding a large

natural lake (350 ha) at daytime. Specimens were found half a meter to a meter above water level on vegetation. The only other amphibian found was *Phrynobatrachus mababiensis*. Additional material was collected at a small pond (<0.5 ha) covered by tick marginal vegetation. Specimens were calling low down on the edge of the open water. Two other *Hyperolius* species were present in the same area, *Hyperolius angolensis* and *Hyperolius* cf. *cinereus*, both species were calling further away and higher up the vegetation. *Hoplobatrachus occipitalis* was present in the pond.

Remarks: The synonymy of *H. sagitta* is supported by the remarkable similarities in the description and the proximity of the type locality within the range of *H. dartevellei*. The species is confirmed genetically from Mozambique, Zimbabwe, Zambia and DRC. Due to the wide distribution and large population numbers, we suggest that this species be regarded as Least Concern.

***Hyperolius friedemanni* sp. nov.** Mercurio & Rödel

Friedemann's Long Reed Frog (Figure 30)

Synonymy: *Hyperolius nasutus* (nec *Hyperolius nasutus* Günther) Mercurio 2011 (part.)

Holotype: SMF 85694 (tissue VM11), an adult male, collected at Karonga, Malawi, 7 February 2007 by V. Mercurio, 9°55'59.6'' S, 33°56'44.6'' N, 472 m a.s.l. Paratypes. ZMB 76095 (tissue VM12), an adult female, with the same details as the holotype; SAIAB 186000, two juvenile specimens (Monkey Bay, Malawi) (Figure 27).

Genetic material: SMF 85694, ZMB 76095 (holotype and paratype) SAIAB 186000 (two specimens) Monkey Bay, Malawi.

Diagnosis: The advertisement call (Figure 34) consists of a brief initial note of eight pulses, followed by six pulses at a slower rate. The duration of the call is 0.12 s. It can be distinguished from species that produce only a buzz, such as *H. acuticeps*, *H. jacobseni* sp. nov., and *H. nasutus*. It can also be distinguished from *H. adspersus*, *H. dartevellei*, and *H. lupiroensis* sp. nov., which produce only a brief single note. It differs from those species with calls longer than 0.2 s, such as *H. benguellensis*, *H. inyangae* sp. nov., and *H. viridis*. It can be

distinguished from those species where the slower part of the call consists of less than half the pulses of the initial note, such as *H. howelli* sp. nov., *H. igbettensis*, and *H. rwandae* sp. nov. Finally, although the structure of the call of *H. poweri* is similar, the two differ in pitch, *H. poweri* having the dominant frequency of 5.9 kHz, while *H. friedemanni* sp. nov. has a dominant frequency of 4.3 kHz. The snout is sharply rounded in profile, which distinguishes it from species with truncated, bluntly rounded, or shark-like snouts; *H. adspersus*, *H. benguellensis*, *H. howelli* sp. nov., *H. igbettensis*, *H. inyangae* sp. nov., *H. jacobseni* sp. nov., *H. poweri*, *H. dartevellei*, and *H. viridis*. It is the only species in the study where the webbing reaches the disc on all toes, at least on one side. This distinguishes it from all other species.

Description of holotype: The width of the gular flap is 5.1, hand 5.5. The top of the snout is flat, with the tip of the snout acutely rounded from above and from the side (Figure 32) (HW/SUL 0.29). The snout is 1.4 times the eye. The tympanum is not visible. The nostrils are positioned near the snout tip (EN/SL 0.5), nostril opening rounded, slightly protruding. Fine teeth are present on the upper jaw. The choanae are small, round. The tongue is long, with the posterior as wide as the length, with the terminal 20% bifurcated. Vomerine processes absent. The hand is 25.5% of the SUL. A small inner metacarpal tubercle is present. The relative finger lengths are 1<4<2<3. The foot is 0.4 of SUL, and the tibia is 0.52 of SUL. The webbing is shown in Figure 33. The skin is smooth on the dorsum and limbs, coarsely granular under the thighs. In preservative the skin becomes transparent. In life the body is pale green, tinged with blue along the legs, and yellow-tipped fingers and toes. There are pale dorsolateral stripes without dark borders that originate at the nostril and run back over the eye to continue to the groin. The iris is golden.

Paratype variation: The female paratype is similar to the holotype. Tympanum not visible. The paratypes from Monkey Bay collected by EN are subadults, with skin that is transparent in preservative, showing large numbers of subdermal parasite eggs.

Advertisement call: Recorded at Karonga, on 7 February 2002 at 23:40 h, 27°C air temperature, voucher specimen SMF 85694. The call (Figure 34) consists of the regular repetition of one single biphasic pulsed note with a duration 110–190 ms. Interval between notes is 180–360 ms. The note repetition rate is 1.4/s. The dominant frequency is 3900–4500 Hz. The specimen was calling at night from dense grassy vegetation within a swamp in an

exposed position about 400 mm above the water. See Table 18 for a summary of call parameters.

Eggs and tadpoles: Unknown.

Habitat: Swamp along the lakeshore with abundant grassy vegetation and sandy soil. Other common species were: *Afrixalus fornasini*, *Hyperolius pusillus*, *H. viridiflavus nyassae*, *H. tuberilinguis*, *Phrynobatrachus acridoides*, *P. mababiensis*, *Ptychadena* cf. *mascareniensis*, *P. anchietae*, *Kassina senegalensis*, *Amietophrynus gutturalis*, *A. maculatus*, *Xenopus muelleri*, *Arthroleptis stenodactylus*, and *Hemisus marmoratus*.

Etymology: We dedicate this new species to Friedemann Schrenk in recognition of his enthusiastic and tireless work for the research and protection of the natural history heritage of Malawi.

Remarks: The species is only known from the shores of Lake Malawi, and we suggest that it be regarded as Data Deficient, in terms of the IUCN criteria.

***Hyperolius howelli* sp. nov.** du Preez & Channing
Howell's Long Reed Frog (Figure 30)

Holotype: SAIAB 118979, collected at Himo Road, Arusha, Tanzania (3°21' 29.6" S; 36°50'15.3" E), collected 12 April 2008 by L. H. du Preez.

Paratypes: SAIAB 118980–1, female, and SAIAB 118980–2, male, collected at Himo Road, near Arusha, Tanzania (3°21' 29.6" S; 36°50'15.3" E), collected 12 April 2008 by L. H. du Preez; NMK 39221 from Kakamega Forest.

Genetic material: SAIAB 118979–80 (Himo Road, Arusha) and a specimen from Madehani, Tanzania (no voucher), NMK 39221 (16S sequence accessioned as AY323926, 12S sequence determined as part of this study) Kakamega Forest, Kenya (Lötters *et al.* 2004) (Figure 27).

Diagnosis: The advertisement call (Figure 36) consists of an initial brief note, followed by three slower pulses, with a duration of 0.12 s. It can be distinguished from species producing

only a single note and those producing only a buzz: *H. acuticeps*, *H. adspersus*, *H. dartevellei*, *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., and *H. nasutus*. It differs from species producing a call over 0.2 s: *H. benguellensis*, *H. inyangae* sp. nov., and *H. viridis*. It differs from those species where the slower, pulsed part of the call has five or more pulses: *H. friedemanni*, *H. igbettensis*, and *H. poweri*. The initial note consists of eight pulses, while the superficially similar call of *H. rwandae* sp. nov. has an initial note consisting of 13 pulses. The shark-like profile of the snout distinguishes it from species with truncated or rounded snouts: *H. acuticeps*, *H. adspersus*, *H. friedemanni* sp. nov., *H. igbettensis*, *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., *H. nasutus*, *H. poweri*, *H. rwandae* sp. nov., and *H. viridis*. The foot has at least one phalanx free of webbing on every toe. This distinguishes it from species where at least one toe is webbed to the disc, at least on one side: *H. adspersus*, *H. benguellensis*, *H. friedemanni*, *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., *H. nasutus*, and *H. rwandae* sp. nov. It also differs from those species that have less than one phalanx free, on at least one toe: *H. acuticeps*, *H. igbettensis*, *H. inyangae* sp. nov., *H. poweri*, *H. dartevellei*, and *H. viridis*.

Description of holotype: Body slender, widest at temporal region, slightly tapering to groin; head comparatively small (HL/SUL 0.32, HW/SUL 0.31), not wider than trunk, slightly longer than wide (HL/HW 1.03); snout top flat, tip of snout rounded (SL/HL 0.48), from above the snout is triangular with a rounded tip (Figure 32), considerably projecting beyond lower jaw with a shark-like profile, wider than long (SL/EE 0.76); canthus rostralis rounded, almost straight-lined from eye to just beyond nostril, slightly convex near tip of snout; loreal region almost vertical, slightly concave; nostril directed laterally; situated much closer to tip of snout than to eye (EN/NS 2.00), separated from each other by distance nearly equal to distance between eye and nostril (NN/EN 1.06); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.36); eye diameter much shorter than snout (ED/SL 0.74); interorbital distance much wider than upper eyelid (IO/EW 0.96), and greater than internarial distance (IO/NN 1.59); tympanum not visible externally; upper jaw with dentition; teeth on premaxilla larger than those on maxilla; choanae small, oval, located far anterolaterally at margins of roof of the mouth; vomer processes and teeth absent; tongue long 4.6, and narrow (2.3 at widest point), free for about three-fourths of length, bifurcated distally for about one-third of length; median lingual process absent; vocal sac single, median, subgular, yellow in colour; gular flap consisting of two areas of thickened skin, the

anterior thicker, cream coloured, and the posterior thinner, smooth and white; vocal sac aperture on each side of the mouth, situated lateral from and close to base of tongue, slit-like, long.

Dorsal surfaces of head, trunk and limbs generally smooth; ventral surface of limbs and gular smooth, chin and abdomen slightly more areolate.

Forelimbs slender; hand moderately large (HND/SUL 0.24); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I< II < IV <III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV, with proximal tubercle on finger IV hardly discernible; webbing formula of the hand I2+–2II2–2.75III2–2.5IV; thenar tubercle indistinct, low; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hindlimbs slender, moderately long (LEG/SUL 1.36); tibio-tarsal articulation passing level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.56), longer than thigh (TFL/THL 1.27); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.70); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1–2II2–1III1–1.5IV1.5–1.25V; inner metatarsal tubercle small, oval, prominent; outer one indistinct.

Colouration in life: Holotype was a brown-green overall, with white lateral stripes running from the snout, through the top of the eye, to the groin. The lateral stripe is lined with irregular large melanophores. The top of each eye has a smudge of golden brown. The back has many small dark melanophores, with a few irregularly spaced larger pigment cells. The limb joints are pale green, with the limbs showing a brown tinge. The fingers and toes are green with yellow tips. The skin is smooth above and on the limbs, while the venter is rough with large flat granules.

Colouration in preservative: The dorsal pattern shows two pale lateral stripes edged with large dark melanophores, filled with opaque white pigment. The head and dorsum is uniformly speckled with small melanophores, with a few irregularly spaced larger pigment cells. A thin dark line runs from the nostril to the eye.

Paratype variation: The female has a similar body shape to the holotype. Skin texture the same as the holotype. Colour in preservative: pale yellow background with large irregular melanophores on the dorsum, overlaying a uniform fine speckling. A dark line runs from eye to eye below the snout tip, running through the nostril. In life the body is pale green with yellowish sides, with darker leaf green around the eyes. The top of the eye has a brown smudge. The line running from eye to eye below the snout tip is reddish brown, with a faint brown band around the top of the snout. The irregular large black spots are less dense posteriorly. The tibia has many large melanophores, with very small speckles on the forearm. The snout profile is rounded, with the nostrils behind the tip. Paratype measurements are included in Appendix 8.

Advertisement call: The call is a harsh insect-like chirp. Males call from elevated positions on vegetation (Figure 31). See Table 18 for a summary of call parameters.

Eggs and tadpoles: Lötters *et al.* (2004) found egg clutches attached to submerged vegetation. The larvae are omnivorous, found in quiet water.

Habitat: The type locality was a pond of roughly 20 m x 40 m with deep clear water. Along the periphery were dense stands of *Typha* sp. where the frogs were present from water level to about one meter above water level. Other species present included *Amietia angolensis*. In Kakamega, *H. cinnamomeoventris*, *H. kivuensis*, *H. lateralis*, and *H. viridiflavus* were present (Lötters *et al.* 2004)

Etymology: We have pleasure in honouring Kim M. Howell for his contributions to East African zoology, made during a long career at the University of Dar-es-Salaam.

Remarks: The species is known from western Kenya, and southern and northern Tanzania. Due to its wide range and large populations, we suggest that it be regarded as Least Concern in terms of the IUCN criteria.

Hyperolius igbettensis Schiøtz, 1963

Igbetti Long Reed Frog (Figure 38)

Genetic material: Two samples without vouchers, and ZMB 76542–43 (Lamto, Ivory Coast);

ZMB 77415 (Kérouane, Guinea); ZMB 77416 (Konsankoro, Guinea); ZMB 77410 (Dantilla, Guinea) (Figure 27).

Diagnosis: The advertisement call (Figure 36) consists of an initial brief note with 12 pulses, followed by five slower pulses, with a duration of 0.12 s. It can be distinguished from species producing only a single note, and those producing only a buzz: *H. acuticeps*, *H. adspersus*, *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., and *H. nasutus*. It differs from species producing a call over 0.2 s; *H. benguellensis*, *H. inyangae* sp. nov., and *H. viridis*, and from those where the initial note consists of less than 10 pulses: *H. friedemanni*, *H. howelli*, and *H. poweri*. The snout is bluntly round in profile, which distinguishes it from species with truncated, shark-like, or sharply rounded profiles: *H. acuticeps*, *H. adspersus*, *H. benguellensis*, *H. dartevellei*, *H. friedemanni*, *H. howelli*, *H. inyangae* sp. nov., *H. lupiroensis* sp. nov., *H. nasutus*, and *H. rwandae* sp. nov. The foot is webbed with one or more phalanges free of web on the first four toes, and half free on the fifth toe. This distinguishes it from species where at least one toe is webbed to the disc, at least on one side: *H. adspersus*, *H. benguellensis*, *H. friedemanni*, *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., *H. nasutus*, and *H. rwandae* sp. nov. It differs from species that have at least one phalanx free on the fifth toe; *H. acuticeps*, *H. dartevellei*, *H. howelli*, *H. inyangae*, and *H. nasutus*. Finally, it differs from the two species that have one or less phalanges of the second toe free of web: *H. poweri* and *H. viridis*.

Description of a Dantilla specimen: This is based on ZMB 77410, an adult female. The ranges are given from three specimens (ZMB 77410–412; 1 female, 2 males), with single measurements from the sequenced specimen. Elongate, fragile frogs; body long and slender (SUL 21.1), widest at temporal region (HW 5.8–7.0; 7.0), slightly tapering to groin; head comparatively small (HL/SUL 0.33, HW/SUL 0.26), not wider than trunk, longer than wide (HL/HW 1.27); snout long (SL/HL 0.43), subelliptical in dorsal view and protruding in lateral view (Figure 32), projecting beyond lower jaw, wider than long (SL/EE 0.73); canthus rostralis indistinct, roundish, straight-lined from eye to just beyond nostril, slightly convex near tip of snout; loreal region almost vertical, slightly concave; nostril round, directed dorsolaterally; situated much closer to tip of snout than to eye (EN/NS 1.91), separated from each other by distance slightly less than distance between eye and nostril (NN/EN 0.90); eyes directed anterolaterally, protruding, relatively small (ED/HL 0.29); eye diameter shorter than snout (ED/SL 0.67); interorbital distance narrower than upper eyelid (IO/EW 0.8), and greater than

internarial distance (IO/NN 1.11); tympanum barely visible, very small with tympanum-eye distance equal to half diameter of eye; upper jaw with dentition; choanae small, oval, located far anterolaterally at margins of roof of the mouth, concealed by upper jaw for about the half in ventral view; vomer processes and teeth absent; tongue long 5.0, and wide (3.6 at widest point), free for about three-fourths of length, bifurcated distally for about one-fourth of length; median lingual process absent; vocal sac single of males, median, subgular, mostly unpigmented and translucent when fully inflated; gular gland large covering 2/3 to almost entire throat, dilatable skin visible posterior to gland; width of male gular flap 3.6–4.8; gular flap consisting of two medially arranged, heart shaped and triangular areas of thickened skin, immediately adjacent to each other; anterior, heart shaped, light yellow, larger, more granular, and thicker than posterior, triangular white-coloured part; in resting position only anterior part visible from ventral.

Dorsal surfaces of head, trunk and limbs generally smooth; ventral surface of limbs and gular smooth, lower belly slightly more areolate; a few warts in angle of mouth; supratympanic fold absent.

Forelimbs slender; hand moderately large (HND/SUL 0.28); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: 1<2<4<3; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV, with proximal tubercle on finger IV hardly discernible; finger webbing reaches the proximal subarticular tubercle between fingers 2 and 3, and between 3 and 4, with only traces of webbing on the other fingers; thenar tubercle indistinct, low; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hindlimbs slender, moderately long (LEG/SUL 1.58); tibio-tarsal articulation almost reaching to level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.58), longer than thigh (TFL/THL 1.13; TFL 10.7–11.0); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.86; FOT 10.5–15.3); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) $I1^{+}$–1.25II0.25–2III2–1IV1–0.3V; no visible internal or external metatarsal tubercles.

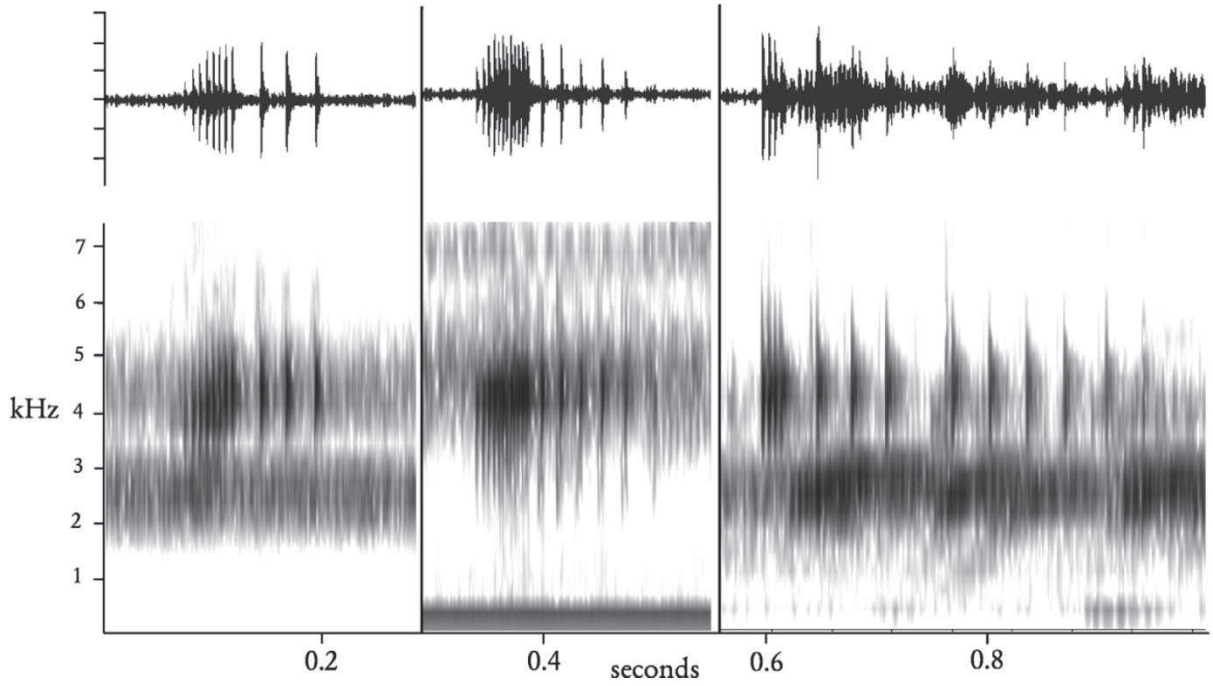

Figure 36. Advertisement calls of *H. howelli*, Arusha (left); *H. igbettensis*, Comoé National Park, Ivory Coast (center); and *H. inyangae*, Rhodes Dam (right).

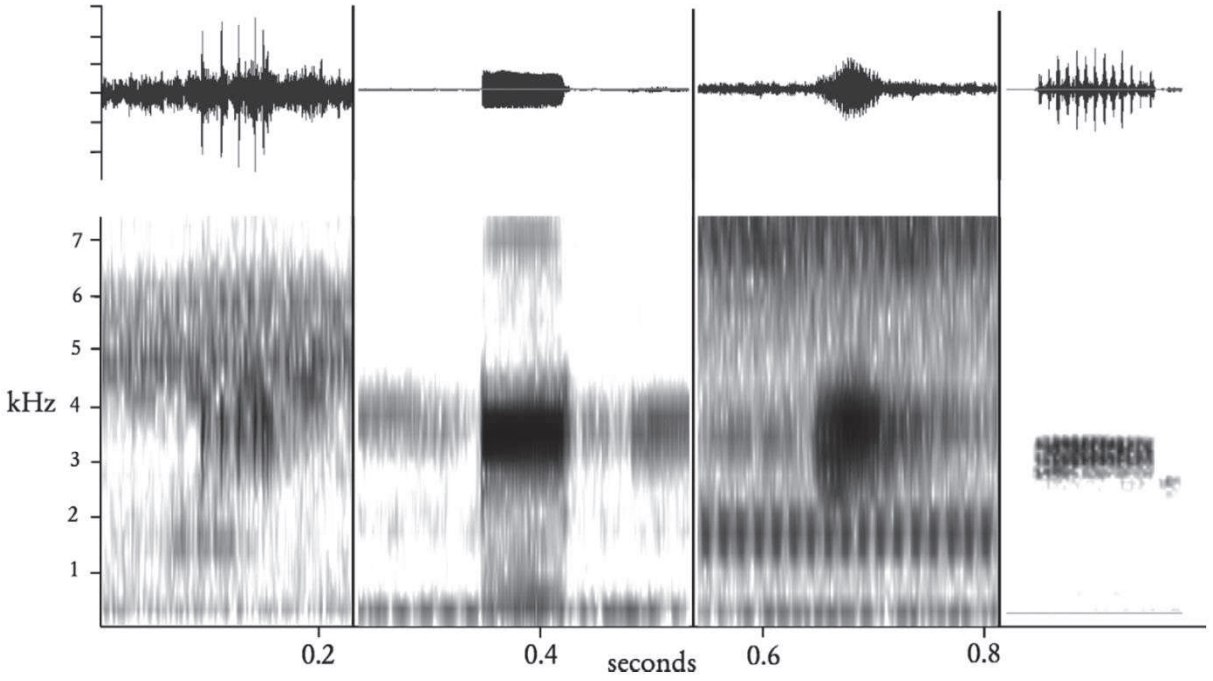

Figure 37. Advertisement calls of *H. jacobseni*, Gatiko (left); *H. lamottei*, Nimba (center left); *H. lupiroensis*, Lupiro (center right); and *H. nasicus*, Fungurume (right).

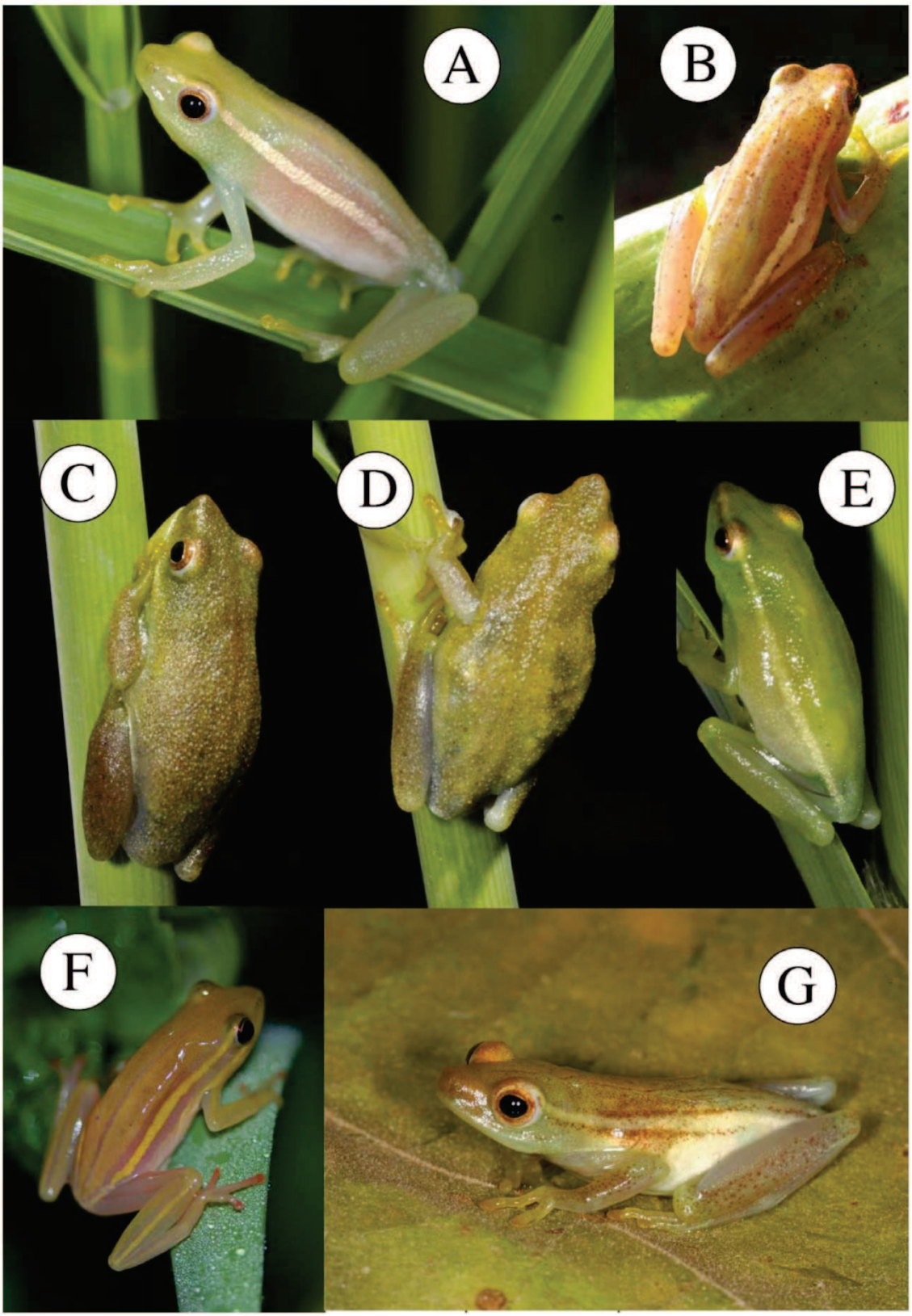

Figure 38. (A) *Hyperolius igbettensis*, Lamto, Ivory Coast, photo M.-O. Rödel; (B) *Hyperolius jacobseni* sp. nov. holotype, ZMB 77280, Gatiko, Central African Republic, photo N. Jacobsen; (C) *Hyperolius inyangae* sp. nov. male holotype, ZMB 77276, Rhodes Dam, Nyanga National Park, Zimbabwe, photo A. Channing; (D) *H. inyangae* sp. nov., female paratype, ZMB 77277, same details; (E) *Hyperolius nasicus*, Nyanga Flats, Zimbabwe, photo A. Channing; (F) *H. lamottei*, night pattern, Mt Nimba, Guinea, photo M.-O. Rödel; (G) *Hyperolius lupiroensis* sp. nov., holotype ZMB 77300, Lupiro, Tanzania, photo A. Channing.

Colouration in life: The basic colour of live frogs ranges from a light bluish green, to grass green or almost green-brown; shanks, lower and upper arms are almost transparent blue-green; flanks, back and thighs darker green with many small dark spots, sometimes arranged along vertebral line into a broken line; eyelids usually lighter than rest of head and dorsum, yellowish to reddish brown; sometimes head darker (reddish brown) than rest of dorsal surfaces; in some animals, mostly males, light white to yellow dorsolateral stripe, rarely bordered by two dark lines; dark canthal stripe, reddish iris bordered by narrow blue line; gular gland of males yellowish or like rest of vocal sac skin light green-blue; ventral surfaces light, belly whitish, often almost transparent; toe and finger tips yellow to orange; females are usually more "transparent" than males, with eggs visible through the body wall.

Colouration in preservative: Very pale beige in preservation with small dark spots scattered over back and extremities, with or without a distinct white dorsolateral band.

Eggs and tadpoles: Unknown.

Remarks: The biology of this species was discussed by Rödel *et al.* (2006). The species is now known from Guinea to Cameroon, and perhaps occurs further east (Amiet 2006a). We suggest that the IUCN status of Least Concern be maintained.

***Hyperolius inyangae* sp. nov**. Channing

Nyanga Long Reed Frog (Figure 38)

Holotype: ZMB 77276, a male, collected at Rhodes Dam in the Nyanga National Park, Zimbabwe, 18°17'20.3" S, 32°43'24.4" E, 14 November 2009.

Paratypes: A female, ZMB 77277, and two males, ZMB 77278–9, with the same collecting details as the holotype.

Genetic material: ZMB 77277–8, ZMB 77276 (Rhodes Dam, Nyanga National Park, Zimbabwe); ZMB 76099-101 (Kaningina, Malawi).

Diagnosis: The advertisement call (Figure 36) consists of a brief initial note of four pulses, followed by nine slower pulses, with a duration of 0.35 s. It can be distinguished from

species producing only a single note, and those producing only a buzz: *H. acuticeps*, *H. adspersus*, *H. dartevellei*, *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., and *H. nasutus*. It can be distinguished from those species with short calls under 0.2 s: *H. friedemanni*, *H. howelli*, *H. igbettensis*, *H. poweri*, and *H. rwandae* sp. nov. It differs from *H. viridis*, which has an initial note consisting of 26 pulses. See Table 18 for a summary of call parameters. It has a shark-like snout profile, which distinguishes it from those species that have truncated or rounded snouts; *H. acuticeps*, *H. adspersus*, *H. dartevellei*, *H. friedemanni*, *H. igbettensis*, *H. jacobseni* sp. nov., *H. lupiroensis* sp. nov., *H. nasutus*, *H. poweri*, *H. rwandae* sp. nov., and *H. viridis*. The webbing is characterized by three phalanges free of the fourth toe, and two phalanges free of the fifth toe. This distinguishes it from all other species (which have more webbing).

Description of holotype: Body long and slender, widest at mid-body, slightly tapering to groin; head comparatively small (HL/SUL 0.37, HW/SUL 0.29), not wider than trunk, longer than wide (HL/HW 1.27); snout long (SL/HL 0.46), sharply rounded in dorsal view, acute in profile with a distinct protruding tip (Figure 32), considerably projecting beyond lower jaw, wider than long (SL/EE 0.82); canthus rostralis distinct, sharp, almost straight-lined from eye to nostril, slightly convex near tip of snout; loreal region almost vertical, slightly concave; nostril directed dorsolaterally; situated midway between tip of snout and eye (EN/NS 1.0), separated from each other by distance greater than distance between eye and nostril (NN/EN 1.15); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.23); eye diameter shorter than snout (ED/SL 0.51); interorbital distance much wider than upper eyelid (IO/EW 1.14), and greater than internarial distance (IO/NN 1.04); tympanum not visible externally; upper jaw with dentition; teeth on premaxilla larger than those on maxilla; choanae small, slightly oval, located far anterolaterally at margins of roof of the mouth; vomer processes and teeth absent; tongue long 4.6, and narrow (2.9 at widest point), free for about one-quarter of length, bifurcated distally for about one-fourth of length; median lingual process absent; vocal sac single, median, subgular, mostly unpigmented and translucent when fully inflated; gular flap consisting of thickened granular skin, vocal sac aperture on each side of the mouth, situated lateral from and close to base of tongue, slit-like, long, directed posterolaterally.

Dorsal surfaces of head, trunk and limbs finely granular with minute tubercles visible under

magnification; ventral surface of limbs and gular smooth, chin and abdomen slightly more areolate.

Forelimbs slender; hand moderately large (HND/SUL 0.24); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV, with proximal tubercle on finger IV hardly discernible; webbing formula of the hand $I2^{+}$–2II2–3III2.5–2.5IV (after Myers & Duellman [1982]); thenar tubercle absent; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hindlimbs slender, moderately long (LEG/SUL 1.42); tibio-tarsal articulation not reaching to level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.48), subequal to thigh (TFL/THL 0.97); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.85); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1.5–1.5II0.75–2III1.5–3IV3–2V; inner metatarsal tubercle absent; outer one almost circular, flattened.

Colouration in life: Head green, overlain with brown pigment which extends over the back and exposed surfaces of limbs. Tibia reddish-brown. Iris and eyelid pale brown. The vocal sac is pale green.

Colouration in preservative: A yellow-brown background, covered dorsally with a dense speckling of small black and brown melanophores and chromatophores. No pale lateral stripes, pigmentation over snout and head more dense than dorsum. Upper exposed surfaces of limbs and digits pigmented.

Paratype variation: The paratypes are similar to the holotype in measurements (Appendix 8). The two males are similar in proportions, including the sharp protruding snout tip, but both have pale lateral stripes. The female, 21.6 SUL, is gravid, with a mid-body width of 10.2. The female also has a sharp shark-like snout, although it is not as acute as those of the males.

Eggs and tadpoles: A female (ZMB 77277) has enlarged ovarian eggs with a diameter of ca.

1.3. Eggs are darkly pigmented on the animal pole and white on the vegetative pole. Tadpoles are unknown.

Etymology: The species is named for the Nyanga National Park, Zimbabwe.

Remarks: The species is known from the Eastern Highlands of Zimbabwe and northern Malawi. The distribution of this species appears to cover at least 900 km of highlands between the collecting localities. Due to the extensive range we suggest that this species be regarded as Least Concern in terms of the IUCN criteria.

***Hyperolius jacobseni* sp. nov.** Channing

Jacobsen's Long Reed Frog (Figure 38)

Holotype: ZMB 77280, a male, collected near Gatiko, Central African Republic, 5°4'43" N, 20°40'2" E, by N. Jacobsen, 29 August 2006.

Paratypes: A female, ZMB 77281, with the same details as the holotype; 16 males and one female, ZMB 77282–298, collected at the same locality, and within a few days of the holotype.

Genetic material: ZMB 77280–1 (holotype and paratype) (Figure 27).

Diagnosis: The advertisement call (Figure 37) consists of a short buzz with five pulses, with a duration of 0.06 s. This distinguishes it from the species with a single unpulsed note, and those with both an initial note and a series of slow pulses: *H. adspersus*, *H. benguellensis*, *H. dartevellei*, *H. friedemanni*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. lupiroensis* sp. nov., *H. poweri*, *H. rwandae* sp. nov., and *H. viridis*. It can be distinguished from the other species with a buzz call by the number of pulses: 25 pulses in *H. acuticeps*, and eight pulses in H. nasutus. See Table 18 for a summary of call parameters. The snout is bluntly round in profile, distinguishing it from those with truncated, shark-like or sharply rounded snouts: *H. acuticeps*, *H. benguellensis*, *H. dartevellei*, *H. friedemanni*, *H. howelli*, *H. inyangae*, *H. lupiroensis* sp. nov., *H. nasutus*,and *H. rwandae* sp. nov. The toes are webbed with one phalanx of the third and fourth toes free, and the fifth toe webbed to the disc. This pattern distinguishes it from those species that do not have the fifth toe webbed to the disc: *H.*

acuticeps, *H. benguellensis*, *H. dartevellei*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. nasutus*, *H. poweri*, and *H. viridis*. It differs from *H. friedemanni* which has all the toes webbed to the disc, and from *H. rwandae* sp. nov. which has two phalanges of the third toe free. The webbing is similar to that of *H. lupiroensis* sp. nov. Standard measurements of the holotype are compared with the other species in Appendix 8.

Description of holotype: Body long and slender, widest at temporal region, slightly tapering to groin; head comparatively small (HL/SUL 0.34, HW/SUL 0.28), not wider than trunk, longer than wide (HL/HW 1.22); snout long (SL/HL 0.43), bluntly rounded in dorsal view, truncated in profile (Figure 32), not significantly projecting beyond lower jaw, wider than long (SL/EE 0.74); canthus rostralis distinct, rounded, strongly concave from eye to nostril, slightly convex near tip of snout; loreal region almost vertical, slightly concave; nostril directed laterally; situated much closer to tip of snout than to eye (EN/NS 1.6), separated from each other by distance greater than distance between eye and nostril (NN/EN 1.31); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.25); eye diameter shorter than snout (ED/SL 0.58); interorbital distance much wider than upper eyelid (IO/EW 2.9), and greater than internarial distance (IO/NN 1.38); tympanum not visible externally; upper jaw with dentition; teeth on premaxilla larger than those on maxilla; choanae small, oval, located far anterolaterally at margins of roof of the mouth; vomer processes and teeth absent; tongue long 4.7, and narrow (2.8 at widest point), free for about three-fourths of length, bifurcated distally for about one-fourth of length; median lingual process absent; vocal sac single, median, subgular; gular flap consisting of two medially arranged, subcircular areas of thickened skin, immediately adjacent to each other; anterior part cream-coloured, larger, more granular, and thicker than posterior white-coloured part; vocal sac aperture on each side of the mouth, slit-like, long.

Dorsal surfaces of head, trunk and limbs smooth but with many densely and more or less evenly scattered tiny, low, spine-like tubercles; ventral surface of limbs and gular smooth, chin and abdomen slightly more areolate.

Forelimbs slender; hand moderately large (HND/SUL 0.28); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV, with proximal tubercle on finger IV hardly discernible; webbing formula of the hand

I2–2.5II2.5–3III2.5–2IV; thenar tubercle indistinct, low; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hindlimbs slender, moderately long (LEG/SUL 1.60); tibio-tarsal articulation reaching to level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.52), longer than thigh (TFL/THL 1.05); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.78); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1–1II0.25–1III0.25–1IV1–0V; inner metatarsal tubercle small, oval, prominent; outer circular, low and less distinct.

Colouration in life: The body is an overall yellow-brown, with a green tinge visible through the skin of the sides of the body. The lateral stripes are bright white, edged with brownish pigment spots.

Colouration in preservative: Colour in preservative pale yellow, with pigmented snout, a blotch of pigment on top of the eye, and minute black melanophores on the back, more dense anteriorly, with larger brown spots irregularly scattered. White lateral lines run from the top of the eye to the groin, bordered by dark lines of spots and melanophores. The belly is white.

Paratype variation: The paratypes are similar in size and proportions to the holotype, with the large female ZMB 77281 having SUL 19.5 and with HW 6.2, with the largest female having SUL 21.5. The male paratypes have a conspicuous muscle (m. ileolumbaris) running from behind the tympanum to the groin, visible under the skin. The inner metatarsal tubercle is flattened, while the outer metatarsal tubercle is absent. The discs on the toes are slightly wider than the width of the toes.

Eggs and tadpoles: A female paratype ZMB 77281 contains enlarged ovarian eggs with a diameter of ca. 1.1. Eggs are darkly pigmented on the animal pole and white on the vegetative pole. Tadpoles are unknown.

Habitat: The types were found on emergent grass and other plants around temporary pools.

Etymology: This species is named for the collector, the South African herpetologist Niels Jacobsen.

Remarks: The species is only known from southern Central African Republic, although it is probably widespread. It should be regarded as Data Deficient in terms of the IUCN criteria.

Hyperolius lamottei Laurent, 1958
Lamotte's Reed Frog (Figure 38)

Synonymy: *Hyperolius nasutus* (nec *Hyperolius nasutus* Günther) Channing *et al.* 2002 (part.)

Genetic material: ZMB 76536–7(Loma Mountains, Sierra Leone); ZMB 76535 (Nimini Forest Reserve, Sierra Leone); ZMB 76525 (Korombadou/ Tourou, Guinea); ZMB 76532 (Mont Béro Forest Reserve, Guinea); ZMB 76526–27 (Nimba Mountains, Guinea); three samples (no vouchers), (Mare d'hivenage, Nimba, Guinea); ZMB 76516 (Savanne de But, Nimba, Guinea); ZMB 76523–24 (Nimba Mountain, Guinea) (Figure 27).

Diagnosis: It is distinguished on the overall yellow background colour pattern, rounded body shape and advertisement call from the species in the *H. nasutus* clade. The call (Figure 37), recorded at Lamto by Arne Schiøtz, is a brief unpulsed whistle that has a duration of 0.08 s, and a dominant frequency of 3.5 kHz. See Table 18 for a summary of call parameters. It is known mostly from high altitude grassland in Senegal, Sierra Leone, Guinea, Liberia and Ivory Coast (e.g. Rödel & Ernst 2003, Rödel *et al.* 2004, Adeba *et al.* 2010). The night-time colour pattern is shown in Figure 38, and the day-time pattern is illustrated in Schiøtz (1999) and Rödel & Ernst (2003). In our dataset, *H. lamottei* is outside the *H. nasutus* group, with a genetic distance for the 16S fragment of 13.2–16.4% to the species inside the group. Tissues were available from near the type locality.

Description of Nimba material: A male ZBM 76526, from the type locality, Mount Nimba, Guinea. Body long and slender, widest at temporal region, almost parallel to groin; head comparatively small (HL/SUL 0.33, HW/SUL 0.28), not wider than trunk, longer than wide (HL/HW 1.14); snout long (SL/HL 0.44), subelliptical in dorsal view, protruding in profile (Figure 32), considerably projecting beyond lower jaw, almost as wide as long (SL/EE 0.97);

canthus rostralis indistinct, round, very slightly concave from eye to nostril, slightly convex near tip of snout; loreal region almost vertical, slightly concave; nostril directed dorsolaterally; situated much closer to tip of snout than to eye (EN/NS 1.6), separated from each other by distance greater than distance between eye and nostril (NN/EN 1.19); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.38); eye diameter shorter than snout (ED/SL 0.87); interorbital distance almost equalling upper eyelid (IO/EW 0,94), and greater than internarial distance (IO/NN 1.53); tympanum not visible externally; upper jaw with dentition; choanae small, oval, located far anterolaterally at margins of roof of the mouth, completely concealed by upper jaw in ventral view; vomer processes and teeth absent; tongue broad and heart shaped, free for about three-fourths of length, bifurcated distally for about one-fourth of length; median lingual process absent; vocal sac single, median, subgular, mostly unpigmented and translucent when fully inflated; gular flap glandular, white in preservative, with folded skin posteriorly; width of gular flap 4.6, gular flap consisting of one round areas of thickened skin, cream-coloured; vocal sac aperture on each side of the mouth, situated lateral from and close to base of tongue, slit-like, long, directed posterolaterally.

The skin of the dorsum and upper limbs is smooth, with a flat granular belly; supratympanic fold absent.

Forelimbs slender; hand short (HND/SUL 0.18); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV; basal webbing only lacking between fingers I and II; thenar tubercle distinct; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hindlimbs slender, comparatively short (LEG/SUL 1.18); tibio-tarsal articulation almost reaching to level of tip of snout when legs are adpressed to body; tibiofibula short (TFL/SUL 0.41), shorter than thigh (TFL/THL 0.96); heels in contact when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.81); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles indistinct: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1.5–2.5II1–2+III1+–2IV1–1.25V; inner and outer metatarsal tubercle indiscernible.

Colouration in life: The back and flanks are dark beige to yellow and densely covered with minute melanophores, bands of more densely arranged melanophores border broad white dorsolateral bands; the back with a narrow black vertebral line, a white longitudinal band on upper surfaces of tibia, slightly bordered darker, not very distinct. The colour pattern may be either fainter or with much more contrasting darker stripes and lines in different individuals.

Colouration in preservative: All colours fade, but the pattern remains distinct.

Eggs and tadpoles: The eggs were described by Schiøtz (1967). The tadpole was described by Arnoult & Lamotte (1958). See Rödel (2000).

Habitat: Humid savanna habitats close to forest belt, mostly in mountainous areas. Reaching altitudes of above 1000 m a.s.l. Often in areas with low grasses and rocky ground.

Distribution: Recorded from Senegal, though Sierra Leone, Liberia, southern Guinea into western Ivory Cost (Rödel 2000, Rödel & Ernst 2003). A population from Central Ivory Coast, Lamto Reserve, may be extinct (Adeba *et al.* 2010).

Remarks: Channing *et al.* (2002) suggested that this species should be regarded as a junior synonym of *H. nasutus*, based on similarity of colour pattern and advertisement call structure, which view was disputed by Rödel & Agyei (2003) and Schiøtz (2006b). Molecular evidence presented in this paper shows that it is not in the *H. nasutus* species group. The sequences of 13 specimens of *H. lamottei* all group together forming a clade outside the *nasutus* group, with little variation. The intraspecific differences in the sample range from 0.0–0.63% for 16S. The conservation status of this species is Least Concern (IUCN 2011).

***Hyperolius lupiroensis* sp. nov.** Channing

Lupiro Long Reed Frog (Figure 38)

Holotype: ZMB 77299, a gravid female collected near Lupiro, 8°25'29.3" S, 36°41'33.1" E, Ifakara district, Tanzania, by A. Danby, 9 July 2007.

Paratype: ZMB 77300, a subadult male, with the same collecting details as the holotype.

Genetic material: The holotype and paratype (Figure 27).

Diagnosis: The advertisement call (Figure 37) consists of a single unpulsed note, with a duration of 0.06 s. It can be distinguished from the species with calls consisting of a few initial pulses, followed by a number of pulses at a much slower pulse rate, such as *H. benguellensis*, *H. friedemanni*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. poweri*, *H. rwandae* sp. nov., and *H. viridis*. It is different from the longer calls consisting of a number of pulses at a more or less constant rate, such as *H. acuticeps*, *H. dartevellei*, *H. jacobseni*, and *H. nasutus*. See Table 18 for a summary of call parameters. The snout is sharply rounded in profile, which distinguishes it from those species with truncated, shark-like, or bluntly rounded snouts: *H. benguellensis*, *H. dartevellei*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. jacobseni*, *H. poweri*, and *H. viridis*. The toes are webbed with one phalanx of the third and fourth toes free, and the fifth toe webbed to the disc. This pattern distinguishes it from those species that do not have the fifth toe webbed to the disc: *H. acuticeps*, *H. benguellensis*, *H. dartevellei*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. nasutus*, *H. poweri*, and *H. viridis*. It differs from *H. friedemanni* which has all the toes webbed to the disc, from *H. rwandae* sp. nov. which has two phalanges of the third toe free, and from *H. adspersus* which has the fourth toe webbed nearly to the disc.

Description of holotype: Body long and slender, widest at mid-belly, slightly tapering to groin; head relatively small (HL/SUL 0.32, HW/SUL 0.23), not wider than trunk, appreciably longer than wide (HL/HW 1.38); snout long (SL/HL 0.48), bluntly rounded in dorsal view, rounded in profile (Figure 32), slightly projecting beyond lower jaw, longer than wide (SL/EE 1.1); canthus rostralis distinct, rounded, almost straight-lined from eye to nostril; loreal region almost vertical, slightly concave; nostril a thin slit directed dorsolaterally; situated much closer to tip of snout than to eye (EN/NS 2.25), separated from each other by distance almost equal to the distance between eye and nostril (NN/EN 0.94); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.35); eye diameter shorter than snout (ED/SL 0.72); interorbital distance about equal to upper eyelid (IO/EW 0.96), and greater than internarial distance (IO/NN 1.44); tympanum not visible externally; upper jaw with dentition; teeth on premaxilla larger than those on maxilla; choanae small, oval, located far anterolaterally at margins of roof of the mouth; vomer processes and teeth absent; tongue long 4.0, and narrow (2.5 at widest point), free for about three-fourths of length,

bifurcated distally for about one-third of its length; median lingual process absent.

Dorsal surfaces of head, trunk and limbs generally smooth; ventral surface of limbs and gular smooth, chin and abdomen slightly more areolate.

Forelimbs slender; hand moderately large (HND/SUL 0.26); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV, with proximal tubercle on finger IV hardly discernible; webbing formula of the hand I2.5–3II2–3III2.5–2.5IV; thenar tubercle small, distinct; palmar tubercles absent; metacarpals without supernumerary tubercles.

Hindlimbs slender, moderately long (LEG/SUL 1.64); tibio-tarsal articulation passing level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.52), subequal to thigh (TFL/THL 1.03); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.75; relative length of toes: I<II<III<V<IV; discs of toes subequal to those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1–1II0.5–2^{+}III0.25–2IV1–0V; inner metatarsal tubercle small, oval, distinct; outer metatarsal tubercle absent.

Colouration in life: Generally weakly pigmented and skin more or less translucent. Dorsum and dorsal surface of head and limbs yellowish green; lateral sides of head and scapular region light green; light, yellowish white, moderately broad dorsolateral stripe running along each side of the body from lateral edge of upper eyelid to groin, continued as faint, hardly discernible line from eyelid to tip of snout; very small dark brown to black dots and larger brown to reddish brown specks on dorsum, most densely along both sides of canthus rostralis and upper eyelid and to lesser extent on both sides of dorsolateral stripe; dots roundish, specks shaped like stars or neurons with many dendrites; distal portions of fingers and toes, especially the tips, yellow; ventral side and parts of dorsal side of thigh and upper arm largely unpigmented, appearing bluish-green; peritoneum white, shining through the translucent belly skin; most of internal organs covered with silvery-white tissue (only visible when dissected). Iris reddish-brown during the night, yellowish-brown during the day.

Colouration in preservative: All colours have faded to yellow; gular flap whitish-yellow.

Paratype variation: The subadult male is considerably smaller than the type, SUL 14.2, and has bright white lateral stripes originating at the nostrils and running back through the top of the eye to the groin. A dark line runs below the pale band from the nostril to the eye. The pale bands have dark borders between the eye and the groin. The back is uniformly speckled, giving the back a brown colour in preservative.

Eggs and tadpoles: The type contains enlarged ovarian eggs with a diameter of ca. 0.7–0.8. Eggs are darkly pigmented on the animal pole and white on the vegetative pole. Tadpoles are unknown.

Remarks: Although it is common for males to have lateral stripes and the females to be spotted in this group, the gravid holotype shows distinct pale lateral stripes. The species is only known from eastern Tanzania, and should be regarded as Data Deficient in terms of the IUCN criteria.

Hyperolius nasicus Laurent, 1943

Pointed Long Reed Frog (Figure 38)

Genetic material: SAIAB A-136-1 (2 specimens, Elephant's Camp, Mozambique); SAIAB A-188 (Satellite Camp, Mozambique); SAIAB KU95952, KU96401, KU98212 (Quantum Mine & Kalumbila River, Zambia); ZMB 77308 (Kisanfu River, DRC); ZMB 77309 Fungurume, DRC; ZMB 77310 (Nyanga Flats, Zimbabwe); ZMB 77301–2, 77304–7, 77314 Ikelenge, Zambia.

Diagnosis: The advertisement call (Figure 37) consists of a brief chirp-like note of about 10–20 constant-rate pulses with a duration of 0.09–0.2 s. It can be distinguished from species producing only a single note, and those producing only a buzz: *H. acuticeps*, *H. adspersus*, *H. dartevellei*, *H. jacobseni*, *H. lamottei*, and *H. lupiroensis*. It differs from species producing a call over 0.2 s: *H. benguellensis*, *H. inyangae*, and *H. viridis*. It differs from the species that have a number of slower pulses: *H. friedemanni*, *H. igbettensis*, *H. howelli*, and *H. poweri*. The call is similar in structure to that of *H. nasutus*, but the latter call has a shorter duration and a higher pulse rate. See Table 18 for a summary of call parameters. The snout is sharply

rounded from above, with a shark-like tip when viewed from the side, which distinguishes it from those species without truncated, shark-like, or bluntly rounded snouts: *H. adspersus*, *H. dartevellei*, *H. friedemanni*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. jacobseni*, *H. lamottei*, *H. lupiroensis*, *H. nasutus*, *H. poweri*, *H. rwandae*, and *H. viridis*. The first, third and fifth toes have one phalanx free of webbing, distinguishing it from all the other species. Measurements of some individuals are compared with the other species in Appendix 8.

Description of a Nyanga Flats specimen: This is a male (ZMB 77310). Body long and slender, widest at mid-body, slightly tapering to groin; head comparatively small (HL/SUL 0.32, HW/SUL 0.27), not wider than trunk, longer than wide (HL/HW 1.22); snout long (SL/HL 0.45), pointed in dorsal view, acute in profile (Figure 32), with a slightly upturned tip, considerably projecting beyond lower jaw, wider than long (SL/EE 0.78); canthus rostralis distinct, sharply rounded, almost straight-lined from eye to just beyond nostril, slightly convex near tip of snout; loreal region almost vertical, slightly concave; nostril directed dorsolaterally; situated much closer to tip of snout than to eye (EN/NS 1.7), separated from each other by distance equal to distance between eye and nostril (NN/EN 1.0); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.27); eye diameter less than snout length (ED/SL 0.61); interorbital distance subequal to upper eyelid (IO/EW 1.04), and greater than internarial distance (IO/NN 1.41); tympanum not visible externally; upper jaw with dentition; teeth on premaxilla larger than those on maxilla; choanae small, oval, located far anterolaterally at margins of roof of the mouth; vomer processes and teeth absent; tongue long 5.4, and narrow (2.2 at widest point), free for about three-fourths of length, bifurcated distally for about one-third of length; median lingual process absent; vocal sac single, median, subgular; gular flap consisting of thickened skin; anterior part cream-coloured, larger, more granular, and thicker than posterior white-coloured part; vocal sac aperture on each side of the mouth, situated lateral from and close to base of tongue, slit-like, long.

Dorsal surfaces of head, trunk and limbs generally smooth; ventral surface of limbs and gular smooth, chin and abdomen slightly more areolate.

Forelimbs slender; hand moderately large (HND/SUL 0.28); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III

and IV, with proximal tubercle on finger IV hardly discernible; webbing formula of the hand I2–2II2–3III2.5–2.5IV; thenar tubercle indistinct, low; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hindlimbs slender, moderately long (LEG/SUL 1.41); tibio-tarsal articulation not reaching to level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.48), longer than thigh (TFL/THL 1.14); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.84); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1–1II0.5–1III1–1IV1–1V; inner metatarsal tubercle small, oval, prominent; outer one indistinct.

Colouration in life: Generally weakly pigmented. The frog was translucent green, with the pale dorsolateral bands originating at the snout tip. A thin dark middorsal line runs from the snout tip to between the eyes. The iris is dark brown.

Colouration in preservative: The back is pale yellow with dense small dark chromatophores. The pale dorsolateral bands originate at the nostril, run over the eye and continue to the groin. They are not bordered. A darkly pigmented band runs from the nostril to the eye. Ventrally unpigmented.

Eggs and tadpoles: Unknown.

Habitat: This species has been found in open savanna, through densely vegetated areas along the Congo River, to flooded swamp forest. They call from emergent vegetation bordering pools or flowing water up to a meter above the water surface.

Distribution: This species has been confirmed on molecular data from Mozambique, north-western Zambia, eastern Zimbabwe and Democratic Republic of Congo (Figure 27).

Hyperolius nasutus Günther, 1865

Large-nosed Long Reed Frog (Figure 35)

Synonymy: *Hyperolius punctulatus* (Bocage, 1895)

Genetic material: ZMB 77311 (Calandula, Angola); AC2990 (Kangandala, Angola); LdP field specimen (Xigera, Botswana); SAIAB 186001 (Vumbura, Botswana) (Figure 27).

Diagnosis: The advertisement call (Figure 39) consists of a buzz with eight pulses, with a duration of 0.1 s. This distinguishes it from the species with a single unpulsed note, and those with both an initial note and a series of slow pulses: *H. adspersus*, *H. benguellensis*, *H. friedemanni*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. lupiroensis*, *H. poweri*, *H. rwandae* sp. nov., and *H. viridis*. It can be distinguished from the other species that have a buzz call by the number of pulses: 13 pulses in *H. dartevellei*, 25 pulses in *H. acuticeps*, and five pulses in *H. jacobseni*. See Table 18 for a summary of call parameters. The snout is sharply rounded in profile, which distinguishes it from those species with truncated, shark-like, or bluntly rounded snouts: *H. adspersus*, *H. benguellensis*, *H. dartevellei*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. jacobseni*, *H. poweri*, and *H. viridis*. The second to fifth toes are webbed to the disc, or just below the disc. This distinguishes it from those species with half or more of a phalanx of the fourth toe free: *H. acuticeps*, *H. benguellensis*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. jacobseni*, *H. lupiroensis*, *H. poweri*, *H. rwandae* sp. nov., and *H. viridis*.

Description of a Vumbura specimen: A male, SAIAB 186001 has the body long and slender, widest at temporal region, slightly tapering to groin; head comparatively small (HL/SUL 0.28, HW/SUL 0.31), not wider than trunk, longer than wide (HL/HW 0.88); snout short (SL/HL 0.30), rounded to trapezium-shaped in dorsal view (Figure 32), (SL/EE 0.53); canthus rostralis fairly distinct, straight-lined from eye to nostril; nostril oval (0.13 X 0.10), directed dorsolaterally; situated much closer to tip of snout than to eye (EN/NS 1.20), separated from each other by distance greater than distance between eye and nostril (NN/EN 1.58); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.46); eye diameter exceeding than snout length (ED/SL 1.50); interorbital distance much wider than upper eyelid (IO/EW 1.91), and greater than internarial distance (IO/NN 2.32); tympanum not visible externally; upper jaw with dentition; choanae small, oval, located far anterolaterally at margins of roof of the mouth, concealed by upper jaw for about the half in ventral view; vomer processes and teeth absent; tongue long, free for about three-fourths of length, bifurcated distally for about one-fourth of length; vocal sac single, median, glandular, pale

yellow, roughly triangular.

Dorsal surfaces of head, trunk and limbs finely granulated.

Forelimbs slender; hand moderately large (HND/SUL 0.25); tips of fingers enlarged into broad rounded disks; relative finger lengths are I<IV<II<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV, with proximal tubercle on fingers III and IV hardly discernible; webbing formula of the hand $I2^{+}$–2II2.5–2III1.5–2IV; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hindlimbs slender, moderately long (LEG/SUL 1.48); tibio-tarsal articulation reaching to level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.48), longer than thigh (TFL/THL 1.14); foot shorter than tibiofibula (FOT/TFL 0.70); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1–1.5II2–1.5III2.5–2IV2.5—2.5V; inner metatarsal tubercle small, oval, prominent; outer one larger, almost circular, low and less distinct.

Eggs and tadpoles: Unknown.

Colouration in life: Dorsal surface light green, skin more or less translucent. Dorsal surface of body and limbs with very small dark brown to black dots and slightly larger brown to reddish brown specks on dorsum; dots roundish, specks shaped like stars or neurons with many dendrites, limbs green; lateral sides of snout and area above eyes reddish brown, moderately broad dorsolateral stripe running along each side of the body from groin to eye extending to the snout as a thin white line; distal portions of fingers and toes, especially the tips, yellow; ventral side and parts of dorsal side of thigh and upper arm largely unpigmented. Iris reddish-brown.

Colouration in preservative: The back is yellow brown with darker uniform speckles that extend on to the upper surfaces of the limbs.

Remarks: Specimens are similar to the type description, and one was collected from the type locality Calandula (Duque de Bragança). The back is covered with small and large spots. The synonymy of *H. punctulatus* is supported by the spotted back. The species is known from

northern Botswana and northern Angola. Due to the extensive range and large population numbers, we suggest that the IUCN status of Least Concern be maintained.

Hyperolius poweri Loveridge, 1938

Power's Long Reed Frog (Figure 35)

Genetic material: ZMB 77312–3 (Port Edward, South Africa); PEM A 9545–6 (Mkambati Nature Reserve, South Africa) (Figure 27).

Diagnosis: The advertisement call (Figure 39) consists of an initial brief note with seven pulses, followed by five slower pulses, with a duration of 0.12 s. It can be distinguished from species producing only a single note, and those producing only a buzz: *H. acuticeps*, *H. adspersus*, *H. dartevellei*, *H. jacobseni*, *H. lupiroensis*, and *H. nasutus*. It differs from species producing a call over 0.2 s: *H. benguellensis*, *H. inyangae*, and *H. viridis*. See Table 18 for a summary of call parameters. The snout is bluntly rounded, distinguishing it from those with truncated, shark-like or sharply rounded snouts: *H. acuticeps*, *H. benguellensis*, *H. dartevellei*, *H. friedemanni*, *H. howelli*, *H. inyangae*, *H. lupiroensis*, *H. nasutus*, and *H. rwandae* sp. nov. There is a phalanx free of web on the first and third toes, with slightly more than a phalanx free on the fourth toe. The second and fifth toes have about half a phalanx free of web. It can be distinguished from the species that have at least one toe webbed to the disc: *H. adspersus*, *H. benguellensis*, *H. friedemanni*, *H. jacobseni*, *H. lupiroensis*, *H. nasutus*, and *H. rwandae* sp. nov. It differs from the species that have the fifth toe with one or more phalanges free of web: *H. acuticeps*, *H. dartevellei*, *H. howelli*, and *H. inyangae*.

Description of a specimen from Mkambati: This is a male (PEM A 9545), collected at the Mkombati Nature Reserve, Eastern Cape Province, South Africa by J. Venter and W. Conradie, 8 February 2011. Body long and slender, widest at mid-body, slightly tapering to groin; head comparatively small (HL/SUL 0.30, HW/SUL 0.30), not wider than trunk, length subequal to width (HL/HW 0.98); snout long (SL/HL 0.49), sharply rounded in dorsal view, blunt in profile (Figure 32), projecting beyond lower jaw, wider than long (SL/EE 0.75); canthus rostralis distinct, rounded, slightly concave from eye to just beyond nostril, slightly

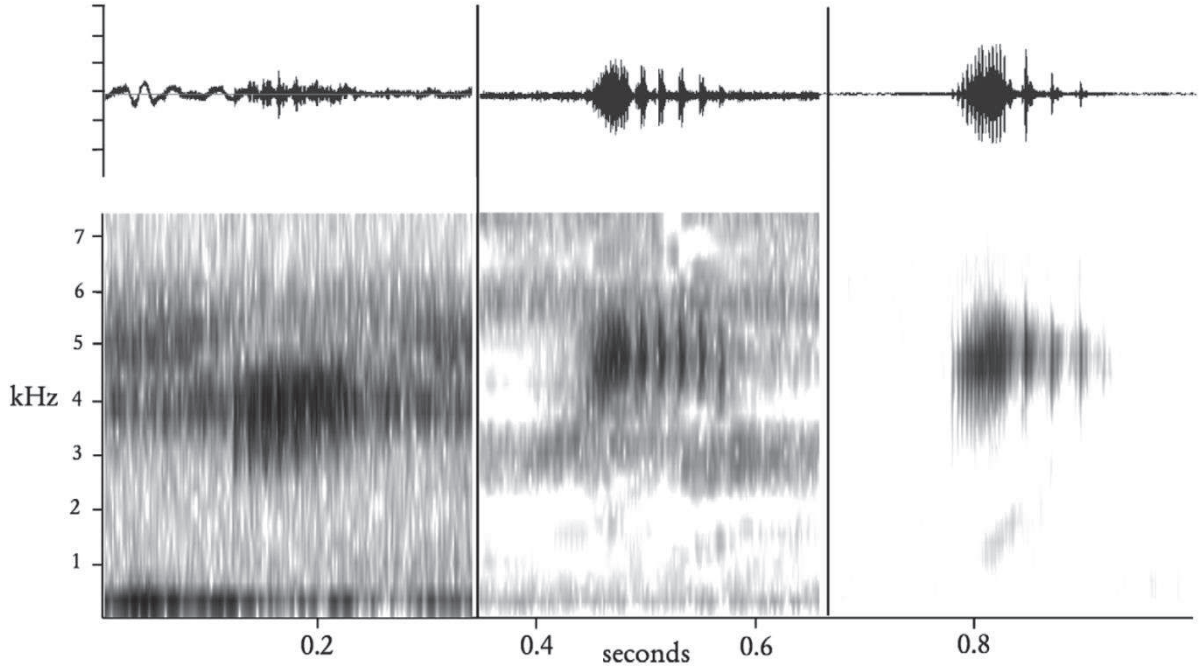

Figure 39. Advertisement calls of *H. nasutus*, Kangandala (left); *H. poweri*, Port Edward (center); and *H. rwandae*, Gitarama (right).

convex near tip of snout; loreal region almost vertical, slightly concave; nostril directed laterally; situated much closer to tip of snout than to eye (EN/NS 1.46), separated from each other by distance greater than distance between eye and nostril (NN/EN 1.16); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.34); eye diameter shorter than snout (ED/SL 0.70); interorbital distance wider than upper eyelid (IO/EW 1.14), and greater than internarial distance (IO/NN 1.09); tympanum not visible externally; upper jaw with dentition; teeth on premaxilla larger than those on maxilla; choanae small, oval, located far anterolaterally at margins of roof of the mouth; vomer processes and teeth absent; tongue long 5.1, and narrow (2.3 at widest point), free for about three-fourths of length, bifurcated distally for about one-fourth of length; median lingual process absent; vocal sac single, median, subgular, mostly unpigmented and translucent when fully inflated; gular flap consisting of two areas of thickened skin, immediately adjacent to each other; anterior part cream-coloured, larger, more granular, and thicker than posterior white-coloured part; in resting position only a narrow band of the posterior part visible from below; vocal sac aperture on each side of the mouth, situated lateral from and close to base of tongue, slit-like.

Dorsal surfaces of head, trunk and limbs generally smooth; ventral surface of limbs and gular smooth.

Forelimbs slender; hand moderately large (HND/SUL 0.27); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV, with proximal tubercle on finger IV hardly discernible; webbing formula of the hand I2–2II2.5–3III3–2.5IV; thenar tubercle indistinct, low; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hindlimbs slender, moderately long (LEG/SUL 1.5); tibio-tarsal articulation passing level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.52), subequal to thigh (TFL/THL 1.04); heels overlapping each other when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.81); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1–1.5II0.75–2III1–2IV1.5–0.5V; inner metatarsal tubercle small, oval, prominent; outer one indistinct.

Colouration in life: In life the body is dark green with pale flecks, and fine brown pigment spots. The lateral stripes are shiny white, with a subdermal paradorsal band visible as an irregular pale green band. The toes have reddish tips.

Colouration in preservative: In preservative the lateral stripes are shiny white, originating at the nostrils, being pale and subdermal before running over the eyes, and extending back to the groin. The back is densely covered in small chromatophores, with very dark pigment over the snout. The gular region is pale with a few dark spots.

Eggs and tadpoles: The eggs are white with a grey animal pole, less than 1 mm in diameter, within capsules, 2.2 mm in diameter (Wager 1986). Clutch size is about 200, with the eggs being deposited in small groups attached to vegetation under water (Wager 1986). Wager (1986) described the tadpoles.

Habitat: The frogs are found on reeds and other emergent vegetation around pools and swamps.

Distribution: This species is only confirmed from the east coast of South Africa, from Mkambati in the south, northwards to the Mozambique border. The northern extent of the distribution is unknown.

Remarks: The species is only known from the north-eastern coastal strip of South Africa. Due to the disturbed coastal habitat, this species should be regarded as Data Deficient in terms of the IUCN criteria, until further studies are carried out.

***Hyperolius rwandae* sp. nov.** Dehling, Sinsch, Rödel & Channing
Rwanda Long Reed Frog (Figure 35)

Holotype: ZMB 77221, adult male, from a pond in farmland on the eastern outskirts of Butare, Huye District, South Province, Rwanda (2°37'10.79'' S, 29°45'08.45'' E), collected 13 September 2010 by J.M. Dehling.

Genetic material: ZMB 77221–2 (Butare, Rwanda); ZMB 77223–4 (Mugesera wetland, Rwanda); ZMB 77225 (Akagera wetland, Rwanda) (Figure 27).

Paratypes: ZMB 77222, adult male, same data as holotype; ZMB 77423–24, 77426–29, six adult males, ZMB 77425, adult female, all from farmland on the eastern outskirts of Butare, Huye District, South Province, Rwanda, collected in October 2009 by K. Lümkemann, K. Rosar and C. Schwartz; ZMB 77686–89, four adult males, from farmland on the eastern outskirts of Butare (2°35'44.1'' S, 29°45'25.6'' E), collected 27 February 2012 by J.M. Dehling; ZMB 77223, adult female, from the Mugesera wetland south of Lac Mugesera, Bugesera District, East Province, Rwanda (2°12'18.92'' S, 30°16'18.18'' E), collected 27 March 2011 by J.M. Dehling; ZMB 77224, adult male, from the Mugesera wetland, Bugesera District, East Province, Rwanda (2°12'15.95'' S, 30°15'49.25'' S), collected 27 March 2011 by B. Dumbo and J.M. Dehling; ZMB 77683 juvenile, ZMB 77684 adult female, ZMB 77685 adult male, all from the Mugesera wetland, Bugesera Province, southeastern Rwanda, collected 26 February 2012 by J.M. Dehling; ZMB 77225, adult male, from a wetland of the Akagera River, Kihere District, East Province, Rwanda (2°13'27.63" S, 30°49'39.06" E), collected 31 March 2011 by J.M. Dehling; ZMB 77746–48, three adult males, from a swamp in farmland on the eastern

outskirts of Ruhengeri, Musanze District, North Province, Rwanda (1°30'25.73" S, 29°39'12.11" E), collected 30 March 2012 by J.M. Dehling.

Diagnosis: The advertisement call (Figure 39) consists of an initial brief note of 13 pulses, followed by three slower pulses, with a duration of 0.14 s. It can be distinguished from species producing only a single note, and those producing only a buzz: *H. acuticeps*, *H. adspersus*, *H. dartevellei*, *H. jacobseni*, *H. lupiroensis*, and *H. nasutus*. It differs from species producing a call over 0.2 s: *H. benguellensis*, *H. inyangae*, and *H. viridis*. It differs from the species that have five or more slower pulses: *H. friedemanni*, *H. igbettensis*, and *H. poweri*. The initial note of the call of *H. howelli* consists of only eight pulses, distinguishing it from *H. rwandae* with 13. See Table 18 for a summary of call parameters. The snout is sharply rounded in profile, which distinguishes it from those species with truncated, shark-like, or bluntly rounded snouts: *H. adspersus*, *H. benguellensis*, *H. dartevellei*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. jacobseni*, *H. poweri*, and *H. viridis*. The third and fifth toes webbed three-fourth the way between disc and distal subarticular tubercle, distinguishing it from the species where the webbing does not reach beyond the distal subarticular tubercles of the third and/or fifth toe: *H. acuticeps*, *H. benguellensis*, *H. dartevellei*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. nasutus*, *H. poweri*, and *H. viridis*. It differs from *H. friedemanni* which has all the toes webbed to the disc and from *H. lupiroensis* and *H. nasutus* which have three phalanges free of web on the inner side of the fourth toe. Standard measurements of the holotype are compared with the other species in Appendix 8.

Description of holotype: Body long and slender, widest at temporal region, slightly tapering to groin; head comparatively small (HL/SUL 0.33, HW/SUL 0.30), not wider than trunk, longer than wide (HL/HW 1.10); snout long (SL/HL 0.44), pointed in dorsal view, acute in profile (Figure 32), considerably projecting beyond lower jaw, wider than long (SL/EE 0.77); canthus rostralis distinct, moderately sharp, almost straight-lined from eye to just beyond nostril, slightly convex near tip of snout; loreal region almost vertical, slightly concave; nostril directed dorsolaterally; situated much closer to tip of snout than to eye (EN/NS 1.42), separated from each other by distance greater than distance between eye and nostril (NN/EN 1.13); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.31); eye diameter shorter than snout (ED/SL 0.70); interorbital distance much wider than upper eyelid (IO/EW 1.71), and greater than internarial distance (IO/NN 1.16); tympanum

not visible externally; upper jaw with dentition; teeth on premaxilla larger than those on maxilla; choanae small, oval, located far anterolaterally at margins of roof of the mouth, concealed by upper jaw for about the half in ventral view; vomer processes and teeth absent; tongue long 4.9, and narrow (2.4 at widest point), free for about three-fourths of length, bifurcated distally for about one-fourth of length; median lingual process absent; vocal sac single, median, subgular, mostly unpigmented and translucent when fully inflated; gular flap consisting of two medially arranged, subcircular areas of thickened skin, immediately adjacent to each other; anterior part cream-coloured, larger, more granular, and thicker than posterior white-coloured part; in resting position only anterior part visible from ventral; vocal sac aperture on each side of the mouth, situated lateral from and close to base of tongue, slit-like, long, directed posterolaterally.

Dorsal surfaces of head, trunk and limbs generally appearing smooth but with many densely and more or less evenly scattered tiny, low, spine-like tubercles, hardly visible with the naked eye; ventral surface of limbs and gular smooth, chin and abdomen slightly more areolate; supratympanic fold absent.

Forelimbs slender; hand moderately large (HND/SUL 0.29); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV, with proximal tubercle on finger IV hardly discernible; webbing formula of the hand $I2^{+}$–2II2–2.75III2–2-IV (after Myers & Duellman [1982]); thenar tubercle indistinct, low; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hindlimbs slender, moderately long (LEG/SUL 1.63); tibio-tarsal articulation reaching to level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.54), longer than thigh (TFL/THL 1.11); heels overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.81); relative length of toes: I<II<III<V<IV; discs of toes smaller than those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I1.5–2^{+}II1.25–2^{+}III1.25–2IV2^{-}–1.25V; inner metatarsal tubercle small, oval, prominent; outer one larger, almost circular, low and less distinct.

Colouration in life: Generally weakly pigmented and skin more or less translucent. Dorsum and dorsal surface of head and limbs yellowish green; lateral sides of head and scapular region light green; light, yellowish white, moderately broad dorsolateral stripe running along each side of the body from lateral edge of upper eyelid to groin, continued as faint, hardly discernible line from eyelid to tip of snout; very small dark brown to black dots and larger brown to reddish brown specks on dorsum, most densely along both sides of canthus rostralis and upper eyelid and to lesser extent on both sides of dorsolateral stripe; dots roundish, specks shaped like stars or neurons with many dendrites; distal portions of fingers and toes, especially the tips, yellow; ventral side and parts of dorsal side of thigh and upper arm largely unpigmented, appearing bluish-green; peritoneum white, shining through the translucent belly skin; most of internal organs covered with silvery-white tissue (only visible when dissected). Iris reddish brown during the night, yellowish brown during the day.

Colouration in preservative: All colours have faded to yellow; gular flap whitish-yellow.

Paratype variation: The paratypes are similar to the holotype in measurements (Appendix 8). Female type specimens (SUL 18.2–20.4, mean 19.2, n=3) are about as large as males (SVL 18.4–22.0, mean 19.5, n=15). Colouration of male paratypes is similar to that of the holotype. In some specimens, however, the pattern of dots and speckles is more pronounced. In others, the lateral stripe is less distinct. The light canthal stripe is completely absent in ten male paratypes and in seven paratypes as faintly visible as in the holotype. All females observed in the field, including the female paratypes, lack the light dorsolateral and canthal stripes, gular sacs and flaps, and the spiny dorsal tubercles (Figure 35). In life, the flanks of the body turn reddish in active males, especially those which are calling.

Eggs and tadpoles: Several females with enlarged ovarian eggs were observed but only three of them were collected (ZMB 77143, 77425, 77684). Their ovaries contain about 80 enlarged eggs with a diameter of ca. 0.7–0.8. Eggs are darkly pigmented on the animal pole and white on the vegetative pole. Tadpoles are unknown.

Habitat: We found the species only in open habitats, in natural wetlands (Mugesera, Akagera) as well as at the edge of ponds and other lenthic water bodies in cultivated areas. Specimens were observed perching on leaves of vegetation between 5 cm and 1.2 m above

the ground or the water level. Males called from elevated positions, sometimes in close proximity to each other (ca. 15 cm). Several males were found engaged in combat. They were holding, pushing, and kicking each other, apparently fighting over an apparently favoured calling site. They also emitted aggressive calls which differed markedly from the advertisement call. The male aggression call is shown in Figure 40. The following species were found sympatrically or even syntopically with the new species: *Afrixalus quadrivittatus*, *Amietia* cf. *angolensis*, *Amietophrynus kisoloensis*, *A. regularis*, *Hyperolius cinnamomeoventris*, *H. kivuensis*, *H. lateralis*, *H. viridiflavus*, *Kassina senegalensis*, *Leptopelis kivuensis*, *Phrynobatrachus* cf. *mababiensis*, *P. natalensis*, *Phrynobatrachus* sp., *Ptychadena anchietae*, *P. porosissima*, *P*. cf. *mascareniensis*, *Ptychadena* sp. and *Xenopus victorianus*.

Distribution: We observed the species at three further locations in Rwanda, near Gitarama (2°05'57.14'' S, 29°46'41.94'' E, Muhanga District, Southern Province, central Rwanda) and west of Kigali (1°57'49.11'' S, 30°00'05.87'' E, Kamonyi District, Southern Province, central Rwanda; and 1°56'59.33'' S, 30°00'48.97'' E, Nyarugenge District, Kigali Province, central Rwanda). The localities from where the species is known are in the northern, central, southern and eastern parts of Rwanda. Elevations of the sites ranged from 1300 m (Akagera wetland) to 1800 m (Ruhengeri). Population size was high at all sites. Because the locations in Butare, Mugesera, and Akagera are only 17 km and 15 km from the border with Burundi and 1.6 km from the border with Tanzania, respectively, and especially because the wetlands of Mugesera and Akagera continue into Burundi and Tanzania, respectively, we assume that the species occurs in these countries as well.

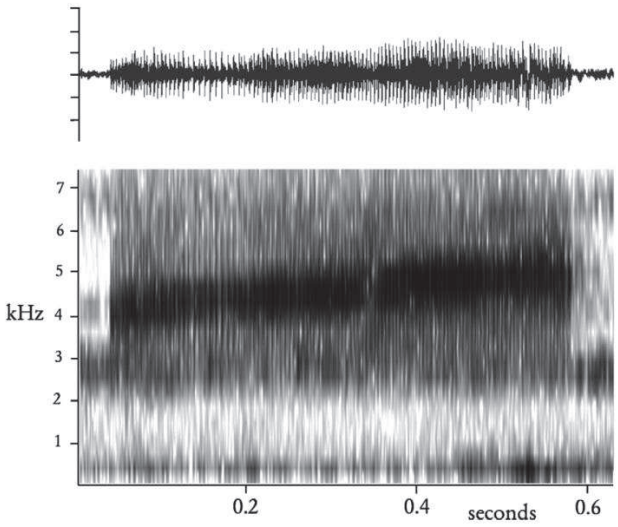

Figure 40. Aggression call of *H. rwandae*.

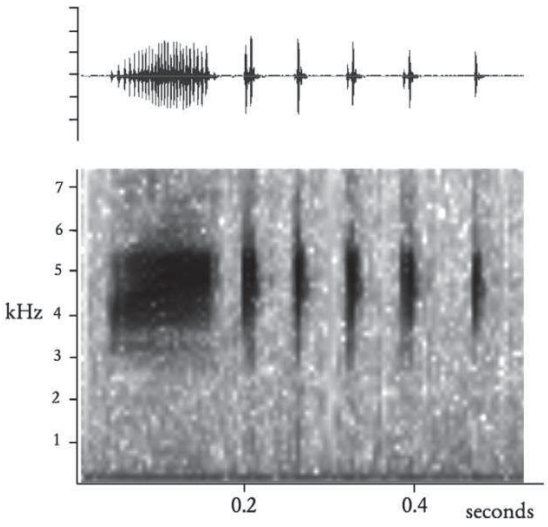

Figure 41. Advertisement call of *H. viridis*, Sumbawanga (A. Schiøtz).

Etymology: The species epithet derives from Rwanda. It is a noun in genitive singular.

Remarks: Although the species is so far only known from several localities in Rwanda, it is probably more widespread. The species occurs in both natural and cultivated areas. Therefore, we propose that it should be classified as Least Concern under the current criteria of the IUCN redlist.

Hyperolius viridis Schiøtz, 1975

Robust Long Reed Frog (Figure 35)

Genetic material: ZMB 76096 (Vintukutu Forest Reserve, Malawi); ZMB 76102 (Kaningina Forest Reserve, Malawi) (Figure 27).

Diagnosis: The advertisement call (Figure 41) consists of a brief initial note consisting of 26 pulses followed by five slower pulses, with a duration of 0.41 s. It can be distinguished from species producing only a single note, and those producing only a buzz: *H. acuticeps*, *H. adspersus*, *H. dartevellei*, *H. jacobseni*, *H. lupiroensis*, and *H. nasutus*. It can be distinguished from the other species producing a two-part call, which have a duration less than 0.4 s: *H. friedemanni*, *H. howelli*, *H. igbettensis*, *H. inyangae*, *H. poweri*, and *H. rwandae*. It differs from *H. benguellensis* which only has five pulses in the initial note. See Table 18 for a summary of call parameters. The snout is bluntly rounded, distinguishing it from those with truncated, shark-like or sharply rounded snouts: *H. acuticeps*, *H. benguellensis*, *H. dartevellei*, *H. friedemanni*, *H. howelli*, *H. inyangae*, *H. lupiroensis*, *H. nasutus*, and *H. rwandae*. The webbing has one phalanx free on the first to third toes, just more than one free on the fourth toe, and half a phalanx free on the fifth toe. It can be distinguished from the species that are webbed to the disc on the fifth toe: *H. adspersus*, *H. friedemanni*, *H. jacobseni*, *H. lupiroensis*, and *H. rwandae*. It differs from the species that have more than half a phalanx free of web on the fifth toe: *H. acuticeps*, *H. benguellensis*, *H. dartevellei*, *H. howelli*, and *H. inyangae*. It can be distinguished from the remaining species that are webbed to the disc on the third or fourth toes: *H. adspersus* and *H. nasutus*. Our specimens show the stocky build noted by Schiøtz (1975).

Description of a Vintukutu specimen. An adult male ZMB 76096, from Vintukutu Forest Reserve, Malawi. Body short and compact, widest at mid-body, tapering to head and neck; head very small (HL/SUL 0.22, HW/SUL 0.34), narrower than mid part of trunk, wider than long (HL/HW 0.64); snout short (SL/HL 0.59), subovoid in dorsal view, almost truncate in profile (Figure 32), only slightly protruding beyond lower jaw, almost as long as wide (SL/EE 0.96); canthus rostralis distinct, rounded, slightly concave between eye to nostril, slightly convex near tip of snout; loreal region almost vertical, slightly concave; nostril directed dorsolaterally; situated closer to tip of snout than to eye (EN/NS 1.18), separated from each other by distance equal to distance between eye and nostril (NN/EN 0.94); eyes directed anterolaterally, moderately protruding, relatively large (ED/HL 0.59); eye diameter equal to snout length(ED/SL 1.0); interorbital distance much narrower than upper eyelid (IO/EW 0.36), but greater than internarial distance (IO/NN 1.3); tympanum not visible externally; upper jaw with dentition; choanae small, round, located far anterolaterally at margins of roof of the mouth, completely concealed by upper jaw in ventral view; vomer processes and teeth absent; tongue slightly longer than wide (2.1), free for about three-fourths of length, bifurcated distally for about one-fourth of length; median lingual process absent; vocal sac single, median, subgular. The gular flap is large (almost completely covering the throat), glandular and wider (4.7) than long (4.1), white in preservative with many minute melanophores.

The skin of the dorsum and upper limbs appears smooth, finely granular under dissecting microscope; flat granular belly; supratympanic fold absent.

Forelimbs slender; hand small (HND/SUL 0.19); tips of fingers enlarged into broad oval disks, each with circummarginal groove; relative length of fingers: I<II<IV<III; subarticular tubercles rounded, well developed, with one on fingers I and II, two on fingers III and IV; only basal webbing between fingers; thenar tubercle oval and prominent; palmar small, round and indistinct; metacarpals without supernumerary tubercles; nuptial pads or asperities absent.

Hind limbs slender, moderately long (LEG/SUL 1.45); tibio-tarsal articulation reaching to level of tip of snout when legs are adpressed to body; tibiofibula moderately long (TFL/SUL 0.54), longer than thigh (TFL/THL 1.09); heels only slightly overlapping each other considerably when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TFL 0.79); relative length of toes: I<II<III<V<IV; discs of toes smaller than

those of fingers; subarticular tubercles: one on toes I and II, two on toes III and V, and three on toe IV; pedal webbing formula (Figure 33) I0.5–1II0.25–1III0.25–1IV1–0.25V; inner metatarsal tubercle small, oval, not very prominent; outer one not discernible.

Colouration in life: The dorsal and ventral surfaces are white, dorsal surfaces (including thighs) densely covered with minute melanophores. Colouration in preservative. All colours have faded to yellow; gular flap whitish.

Eggs and tadpoles: Unknown.

Remarks: The species is known from southern Tanzania and northern Malawi. We suggest that the IUCN status of Data Deficient be maintained until further studies are undertaken.

Incertae sedis

Hyperolius papyri Werner, 1908 is regarded as incertae sedis, as the original description is not sufficiently diagnostic, and we have no fresh material from Southern Sudan for DNA analysis.

Species distribution model

The potential distribution of the *H. nasutus* group (Figure 42) is remarkably similar to the known distribution of the clade (IUCN 2011) comprising major parts of sub-Saharan Africa. The BIOCLIM model suggests a major connected part of the potential distribution in central Africa ranging from southern Cameroon southward to southern Angola and westward to the Kenyan highlands and southward to South Africa. The most humid areas within the central Congo basin and some parts of the West African coast are outside of the occupied climatic space. When interpreting the results, it needs to be acknowledged that the model can only be seen as a rough approximation of the distribution of the clades due to the very limited sample size. The potential distribution describes the climatic space occupied by all members in the group, so the niche of each single species might be much smaller. As soon as additional localities of species can be reliably determined, a model may be refined and computed separately for each species.

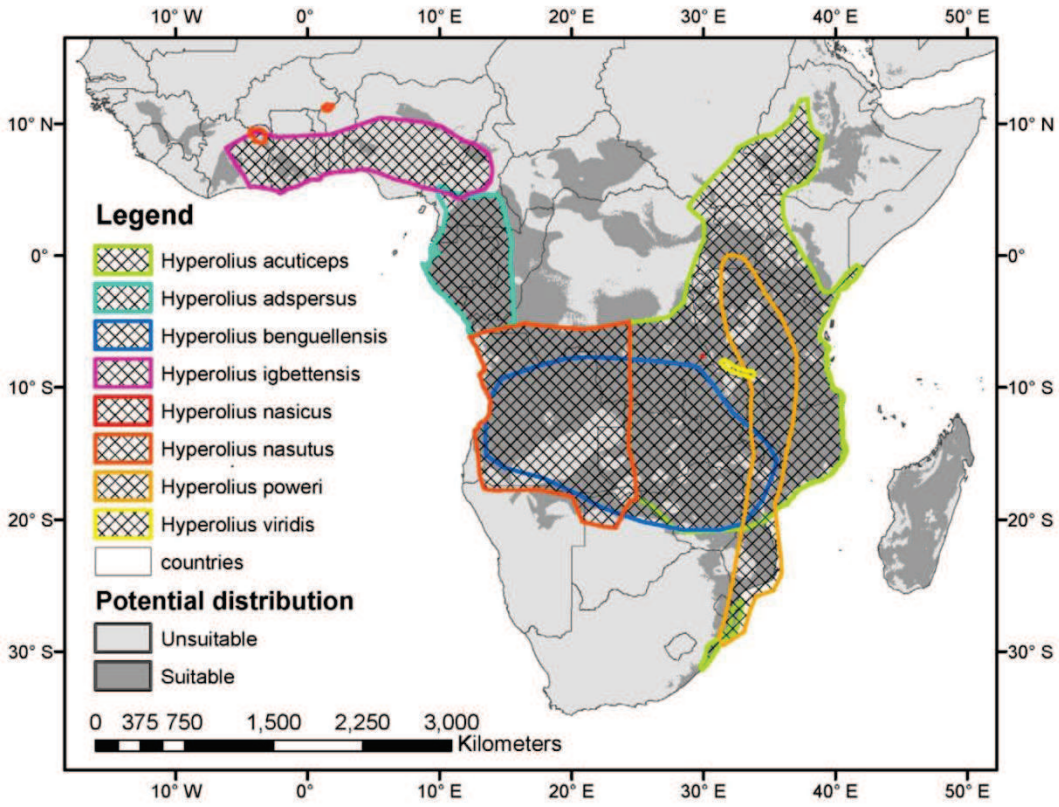

Figure 42. Potential distribution of the *H. nasutus* group derived from a BIOCLIM model. All grey areas are within the environmental envelope of the group, pale areas are outside of the envelope. Overlain is the distribution of the species as recognised by the IUCN (2011). Note that the IUCN taxonomy and species distributions are used purely for illustrating the overall range of the group as known before the present study.

The model (Figure 42) incorporates specimens that extend the range of the group. These are additional records of *H. igbettensis* from Guinea (number 6 in Figure 27) and *H. jacobseni* from the Central African Republic (number 8 in Figure 27).

Discussion

This group of long reed frogs is ubiquitous across the humid to semi-humid tropics of Africa (Figure 42). We suggest that a combination of DNA barcoding, advertisement calls and some aspects of morphology such as webbing and snout shape, is useful in delimiting species. Scientists are univocal that such a pluralistic approach, elsewhere termed 'Integrative Taxonomy', improves our understanding of diversity in an evolutionary framework and helps to identify species. Padial *et al.* (2010) reviewed integration of taxonomically useful characters (i.e. data obtained through independent methodological approaches) via

accumulation versus via congruence and concluded that they can lead to both over- and under estimation of species richness. Because long reed frogs are morphologically difficult to distinguish, we were careful to only recognise a species when molecular in combination with call differences could be shown. Snout shape and degree of webbing provided additional support (Table 17). This approach was termed congruence by Padial *et al.* (2010), leading to the tentative conclusion that our revision of long reed frogs may even underestimate true species diversity in this group. Cryptic species diversity may be present in character sets other than morphology, suggesting that more species await identification.

The polytomies shown in Figure 28 may indicate that some species recognised here split only recently (e.g. Schick *et al.* 2008). In such cases, introgression or incomplete lineage sorting, e.g. with Pleistocene interplay of cold and warm phases, may have occurred (e.g. Noonan & Gaucher 2005; Vences & Wake 2007). The 16S difference between our species is mostly moderate to high (Table 17), with the smallest distances reaching 2.5 % or more, in all but two species pairs. Some individuals show very low to low differences between species. A difference of 3% has been proposed as a rule of thumb as an operable value when distinguishing species using 16S sequences (e.g. Fouquet *et al.* 2007). This applies to most of the species we recognise here (which are otherwise confirmed on the basis of vocalisations, see above). However, it is known that p distances can be much lower between taxa that are otherwise confirmed as 'good' species (Vieites *et al.* 2009; Zimkus & Schick 2010).

There are three kinds of advertisement calls: those consisting of a brief single unpulsed note, those consisting of a pulsed initial note, and a number of slower pulses, and those consisting of a brief chirp. There are many subtle differences between the species, that were not appreciated in an earlier paper that divided the group into three species based on the three kinds of calls (Channing *et al.* 2002). Small differences in the pulse rate of the slower sections, the duration of the call, and the frequency modulation of the call, allow the species as recognised here to have unique advertisement calls. Advertisement call differences are generally associated with species differences. However, the calls of *H. lupiroensis* and *H. lamotteii* overlap in all parameters, despite the significant genetic and morphological differences between these geographically widely separated species. This finding offers interesting opportunities to explore the relationship between speciation and advertisement call divergence.

To strictly operate with integration by congruence and our initial assumption that two or three species can be breeding in the same area, made it imperative that the call and morphology results be based on exactly the specimens that had been sequenced. The exceptions are a call of *H. viridis* from the type locality, when no other recording from a sequenced specimen was available and a call of *H. dartevellei* from the Congo River, that cannot be linked to a sequenced voucher, although all 32 sequenced individuals from there fall into the same species. While we were certain to not mix characters of different species, this procedure limits our study (and species definitions) to a few specimens of each species. This offers opportunities for further work to investigate intra- and interspecific variation in the species recognised here. It is clear that, for example, the within-species variation in characters such as advertisement calls is understated here. Other challenging questions include the geographic distributions of all species, and the niche partitioning of those that are sympatric.

An important implication for future studies of intra- and interspecies variation in long reed frogs concerns the large amount of specimens in museum collections for which no recordings or DNA are available. Many will probably never be positively identified.

This study recognised two species from Ikelenge in north-western Zambia; *H. dartevellei* and *H. nasicus*. This is a positive step in solving what Schiøtz (2006b) called 'the Hillwood mystery'.

Acknowledgements

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thank Arne Schiøtz for helpful comments, and for assistance in obtaining call recordings. Martin Jansen kindly provided pictures, measurements and the webbing formula of the holotype of *H. friedemanni*. Jan Venter of the Eastern Cape Parks and Tourism Agency is thanked for providing logistical and financial support to collect *H. poweri*. JMD would like to thank Antoine Mudakikwa (Rwanda Development Board - Tourism & Conservation) for kindly issuing permissions for field studies, handling and exportation of specimens and Eberhard Fischer (University of Koblenz), Bonny Dumbo (Bukavu, DRC), and Siegmar Seidel (Butare Field Station, University of Koblenz) for logistic support and help during field work.

VII

Controlled aerial descent in the reed frog *Hyperolius discodactylus* Ahl, 1931

This chapter has been published as:

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***Hyperolius discodactylus* (Disc-fingered Reed Frog). Parachuting.** Several species of frogs are capable of a controlled aerial descent (for definition see Dudley *et al.* 2007) which includes gliding flights and parachuting. Traditionally, a gliding flight has been characterized by a descent angle of less than 45° to the horizontal whereas in parachuting, the descent angle is more than 45° (Oliver 1951). The most famous of these species are probably the Southeast Asian flying frogs of the genus *Rhacophorus*, but controlled aerial descent is also known from several other Asian members of the Rhacophoridae, a number of New World hylids, a single member of the Eleutherodactylidae (*Eleutherodactylus coqui*), and a single member of the Hyperoliidae (*Hyperolius castaneus*) (Stewart 1985; Dudley *et al.* 2007; Mendelson *et al.* 2008). During field work in Rwanda in October 2010, I tested the aerial performance of *Hyperolius discodactylus*, a species endemic to the montane forests of the Albertine Rift in Central Africa. I collected four individuals in Nyungwe National Park in southern Rwanda in October 2010 and transported them to a laboratory in the city of Butare. To test their ability to perform parachuting flights, I positioned them at a height of ca. 2 m above the floor and made them jump to the ground by touching their back. Subsequent to the initial leap, the frogs flexed their limbs, spread the fingers and toes and held hands and feet in the frontal plane of the body, which was oriented parallel to the ground (Figure 43). They remained in

this stable posture until they landed on the ground. This posture is known from other species of frogs during controlled aerial descent and has been shown to be best suited for maneuvering in the air (Emerson & Koehl 1990). Two of the frogs were released in the air upside down in a second test. In the air, the frogs turned their bodies by rapid movements of the limbs and quickly assumed the parachuting posture described above. Because the frogs always landed within a horizontal distance of 2 m from the starting point, I choose the term parachuting for the kind of the aerial descent reported here.

Currently, 129 species of *Hyperolius* are considered valid (Frost 2011). Many of these species live in the canopy layer and show morphological characteristics - such as extensive webbing between fingers and toes - that suggest an ability to perform controlled aerial descents. Therefore, controlled aerial descent might be more widespread within *Hyperolius* than currently known.

Figure 43. Parachuting adult male *Hyperolius discodactylus* from Rwanda.

VIII

One or two species? On the case of *Hyperolius discodactylus* Ahl, 1931 and *H. alticola* Ahl, 1931 (Anura: Hyperoliidae)

This chapter has been published as:

H. Christoph Liedtke[1], Dominik Hügli[1], J. Maximilian Dehling[2], Fabio Pupin[3], Michele Menegon[3], Andrew J. Plumptre[4], Deo Kujirakwinja[5] & Simon P. Loader[1] (2014) One or two species? On the case of Hyperolius discodactylus Ahl, 1931 and H. alticola Ahl, 1931 (Anura: Hyperoliidae). – Zootaxa 3768: 253-290.

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Abstract

In 1931, Ernst Ahl described two species of reed frogs inhabiting montane forests of the Albertine Rift in East Africa, *Hyperolius alticola* and *H. discodactylus*, which were synonymized two decades later by Raymond Laurent. Since then, this revision has been questioned repeatedly, but taxonomists have been reluctant to make a conclusive decision on the matter, especially since the type material of *H. alticola* was reported as being lost. Here, we examine the rediscovered type material of *H. alticola* and reassess the validity of Laurent's synonymy using morphological data from historic and new collections including all

available type material, call recordings and molecular data from animals collected on recent expeditions. We find evidence for a northern and southern genetic clade, a divide that is somewhat supported by diverging morphology as well. However, no distinction in advertisement calls could be recovered to support this split and both genetic and morphological differences between geographic units are marginal and not always congruent and thus more likely reflect population-level variation. We therefore conclude that *H. alticola* is not a valid taxon and should continue to be treated as a synonym of *H. discodactylus*. Finally, we also report on newly collected material from outside the species known range, with first records of this species from Burundi.

Key words: Albertine Rift, amphibian, morphology, phylogeny, 16S rRNA, reed frog, sexual dimorphism, advertisement call

Introduction

The taxonomy of the African reed frog genus *Hyperolius* Rapp, 1842 (Anura: Hyperoliidae) has undergone multiple revisions in recent history (Drewes 1984; Richards & Moore 1996; Vences *et al.* 2003; Odierna *et al.* 2007). With 131 currently described species (Frost 2013), it is the most species-rich genus of African anurans, distributed across all of Sub-Saharan Africa. As their vernacular name suggests, these small to medium sized frogs (average snout-vent length of 20 to 35 mm; Channing & Howell 2006) inhabit the edges of permanent or temporary water bodies in forests, savanna and farm bush where males call from reeds protruding from the water's surface. Females fix clutches of eggs to submerged vegetation or leaves overhanging the water, and also to terrestrial vegetation presumably to evade aquatic larval predators (Vonesh 2005). Where known, males are often brightly coloured with species showing sexual dimorphism (Schiøtz 1999) and dichromatism (Schiøtz 1999; Veith *et al.* 2009; Bell & Zamudio 2012). Due to a combination of conserved interspecific morphology and extreme intraspecific variation in colouration (e.g. more than 40 different colour morphs are recognized for the *Hyperolius viridiflavus* complex; Channing & Howell 2006), species identification can be difficult. Furthermore, most pigmentation appears to be soluble in alcohol and thus is usually absent from museum specimens (Laurent 1950b) making it an even less reliable characteristic when working with preserved material. Some

authors have even gone so far as to state that "a workable, yet accurate key is impossible to construct for this genus" (Schiøtz 1975). Given their diversity, hyperoliids are important species for examining evolutionary and biogeographic questions. Having a solid taxonomic foundation for hyperoliids is therefore a priority for addressing such questions and complementing classic morphological studies with molecular techniques has allowed considerable progress to be made (Rödel *et al.* 2010; Schick *et al.* 2010; Dehling 2012a; Channing *et al.* 2013; Conradie *et al.* 2013). This study aims to clarify the taxonomy of the synonymized species *Hyperolius discodactylus* Ahl, 1931 and *H. alticola* Ahl, 1931.

Taxonomic history of H. discodactylus *and* H. alticola

In 1931, Ernst Ahl, who at the time was the "Wissenschaftlicher Oberassistent" of the department of Ichthyology and Herpetology in the Museum für Naturkunde in Berlin (Paepke 2013), announced the description of 87 species of *Hyperolius* solely from preserved specimens in "Das Tierreich", published in March 1931 (Ahl 1931a). Species descriptions by Ahl are notoriously difficult to work with due to a lack of consistency and coherence in the structuring of his taxonomic accounts. Species accounts often lack basic information such as sex, morphometric data beyond snout-vent length, precise locality information and even voucher numbers. Ahl's species descriptions have frustrated his successors, for example, Laurent (1961) exclaimed: "it would be desirable to declare all descriptions resulting of the activity of this singular zoologist as nomina confusa… …[because] Ahl had no idea what variability of a population means, sometimes describing as many as five or six species from specimens belonging to one perfectly homogeneous sample".

H. discodactylus and *H. alticola* are two of the hyperoliids described in "Das Tierreich" (Ahl 1931a). Since their description, the validity of both *H. discodactylus* and *H. alticola* has been repeatedly questioned, and although *H. alticola* is currently considered a synonym of *H. discodactylus* (Frost 2013), the two names have been used interchangeably in the literature (e.g. *H. discodactylus* in Loveridge 1957; Laurent 1972; Channing & Howell 2006; Dehling 2012a; 2012b; and *H. alticola* in Schiøtz 1975; Drewes & Vindum 1994; Schiøtz 1999; Wieczorek *et al.* 2000; Drewes & Wilkinson 2004; Dehling 2012a). The uncertain taxonomic status of these taxa is further confounded by the reported loss of the type material of *H. alticola* (Schiøtz 1975; Frost 1985). Therefore, the validity of the synonym (Laurent 1961; Schiøtz 1975), the revision of which most likely was done without examination of the type

material (see below), is difficult to assess.

In 1931, Ahl provided short accounts on *H. discodactylus* and *H. alticola*, two new species from East Africa, in "Das Tierreich" (Ahl 1931a). A few weeks later, he published more detailed descriptions of the two taxa in "Mitteilungen aus dem Zoologischen Museum in Berlin" (Ahl 1931b). The accounts are brief, but still provide some qualitative detail concerning head and body shape as well as webbing and toe and finger disc shape. However, despite their obvious similarities, Ahl made no effort to draw comparisons between the two and the binomial key on hyperoliids provided (Ahl 1931b) contains little useful information to separate them. No morphometric details are provided other than body size, no reference to voucher numbers is made and the sexes of the specimens are not stated either.

The description of *H. discodactylus* has page priority (p. 363 before p. 379; Ahl 1931a) and was based on seven individuals collected from Rugege Forest (now Nyungwe Forest), Rwanda, and "west of Lake Albert-Edward" (now Lake Edward) in Virunga National Park, Democratic Republic of the Congo (DRC), with Nyungwe Forest being designated as the type locality. All seven specimens appear to have been collected by R. Grauer between 1907 and 1908 during the "German Central Africa Expedition" (Schubotz 1913; Ahl 1931b). The first entry of this species in the museum catalogue in Berlin was made some 20 years later, most likely under the curatorship of Heinz Wermut in the 1940s or 50s, following the advice of Laurent (see below). The entry reads "*Hyperolius discodactylus*, ZMB 36089, one individual from Nyungwe Forest". There is no mention of the other six specimens.

In an inventory of type material/specimens of the Museum of Comparative Zoology (MCZ) collection in Harvard a cotype (syntype) of *H. discodactylus* is listed (MCZ A-17634). The MCZ A-17634 *H. discodactylus* specimen was on exchange from Berlin in 1932 (Barbour & Loveridge 1946). The specimen, collected west of Lake Edward, appears to be one of the seven original types mentioned by Ahl (1931a).

The description of *H. alticola* was based on two specimens collected on the Rwenzori Mountains at 1800 m a.s.l. Although not explicitly stated, it is plausible that they were also collected by Grauer on the same expedition. Both specimens are listed as types, but again, no information is provided as to specimen numbers, sex or more precise locality information. The first catalogue entry was made considerably later than that of *H. discodactylus*, most likely in the 1970s, and the entry assigns both specimens to one number,

Table 19. Summary and comparison of Ahl's (1931b) species descriptions. Traits in bold differ between species.

	H. discodactylus	*H. alticola*
Build	Robust	Robust
Body Length	34mm	37mm
Vomerine Teeth	Absent	Absent
Posterior Nasal Apertures	Small, in part hidden beneath the jaw edges	Not particularly large, in part hidden beneath the jaw edges
Tongue	Small, pear-shaped, free at the back and with two tips	Large, free at the rear with two tips
Head	Large, wider than long. Snout tapered in profile, not extending beyond the mouth.	Large, wider than long, snout pointed and does not (or barely) extend past the mouth
Canthus Rostralis	Distinct and straight	Distinct and straight
Loreal Region	Not mentioned	Steep and concave
Nostrils	Closer to snout tip then to eye	Closer to snout tip then to eye
Internasal Distance	0.5 as wide as the interorbital space	Narrower than interobrital space
Interorbital Space	2x as wide as top eyelid	1.5x as wide as the top eyelid
Tympanum	Hidden beneath a thin layer of skin	Free, small
Finger Webbing	0.5 of fingers webbed	Two outer fingers 0.5 webbed
Finger Discs	Large (out of the ordinary)	Large and sturdy
Finger Length	1st finger shorter than 2nd, 2nd shorter than 4th, 4th only slightly shorter than 3rd. 3rd finger much longer than the snout	1st finger shorter than 2nd, 2nd only slightly shorter than 4th, 3rd finger 1x diameter of a disc longer than the 4th, 3rd finger longer than the snout
Toe Webbing	1/3 of toes webbed. 4th toe, 1.5 phalanges free, the rest 0.5 phalanges free.	3/4 of toes webbed. 4th toe. 1-1.5 phalanges free. Rest of the toes webbed up to or almost up to the discs.
Toes Discs	Somewhat smaller than finger disc	Smaller than finger disc
Toe Length	5th toe barely longer than 3rd	5th toe only slightly longer than 3rd
Outer Toe	No skin fold	Not mentioned
Metatarsus and Tarsus	No tarsal fold, no tarsal tubercle, only a small, flat inner metatarsal tubercle	No tarsal fold, no tarsal tubercle, only a small, flat inner metatarsal tubercle
Tibiotarsal Articulation	Reaches front edge of eye	Reaches middle or front edge of eye
Femur Length	Shorter than tibia	Shorter than tibia
Tibia Length	3.5-4x as long as wide, 0.5 the length of the body and longer than the foot.	4-4.5 times as long as wide, 0.5 the length of the body and shorter than the foot
Limb Proportions	Heels overlap when folding in legs	Heels overlap when folding in legs
Subarticular Tubercle	Small and barely protruding	Very visible
Other Tubercles	Only very small, flat, inconspicuous Medial tubercle (i.e. On inner toe). No metatarsal etc. Tubercles	Only very small, flat, inconspicuous Medial tubercle (i.e. On inner toe). No metatarsal etc. Tubercles
Colouration in ethanol	Dosrum and flanks brownish-white with densely speckled darker pigments flowing together to form a canthal line. Ventrum unicoloured pale yellow.	Unicoloured, pale yellow dorsum and flanks, bright yellow ventrum and underside of extremities. Dark canthal line extending through the eye to the tympanum. Dark spots on upper lip
Skin: Dorsum and Head	Dorsum and sides of head speckled with fine, pointy tubercles. Behind the corner of the mouth and near tympanum, larger warts.	Only fine tubercles between the eyes (sometimes) and larger warts behind the corner of the mouth
Skin: Venter	Coarsely granulated, clear postpectoral fold, temporofemoral fold not very visible. Males with sub-gular vocal sac	Coarsely granulated thighs and belly, no post-gular, post pectoral or temporofemoral folds.

ZMB 39008. In 2009, a second number, ZMB 74944, was designated to the specimens, but not to one specifically.

When comparing the two descriptions, *H. discodactylus* appears to be slightly smaller and more slender with vermiculations on the dorsum and less extensive toe webbing than *H. alticola*, which also boasts a dark line accentuating the canthus. Although there are indications of clear morphological differences, it is unclear to what extent these just reflect intraspecific variability, sexual dimorphism or artefacts of preservation. The description of *H. discodactylus* was potentially based only on males (although five specimens of unknown sex are missing) whereas the description of *H. alticola* was most certainly based only on females. Table 19 summarizes and compares Ahl's descriptions of the two species.

Raymond Laurent was the first reviser and suggested the synonymy of *H. alticola* with *H. discodactylus* in 1947. He reported on the collected material of G. F. de Witte from an expedition to Virunga National Park between 1933 and 1935 and wrote: "the following synonymies are proposed, justified by considering many series" (Laurent 1947). No further information is provided and presumably *H. discodactylus* was chosen over *H. alticola* based on page priority of the original description. In 1950, Laurent described the morphology of eight specimens collected by de Witte from near Mokoto Lakes and Kibaga (south of Mt. Visoke) in DRC and Kundhuru-ya-Tshuve in northwestern Rwanda and compared them with material from the British Museum of Natural History from Kayonsa Forest (now Bwindi Impenetrable National Park) and an individual collected from the Rwenzori Mountains by Loveridge (Laurent 1950b). He provided a table of measurements, colour plates by Laurent with photographs by de Witte and notes on geographic variations in digit length and internarial distance. He also discussed the morphological similarity to other *Hyperolius* species including *H. boulengeri* Laurent, 1943 (currently considered a synonym of *H. phantasticus* [Boulenger, 1899]), *H. koehleri* Mertens, 1940 and *H. castaneus* Ahl, 1931. However, it remains entirely unclear how he justified the synonymy three years earlier.

In 1961, Laurent published a note on the *Hyperolius* and *Afrixalus* specimens of the museum in Berlin (Laurent 1961) and referred to ZMB 36089, a male from Nyungwe forest, as the holotype of *H. discodactylus*. This is presumably where the original catalogue entry stems from. He commented that this specimen corresponded well to what he had formerly been considering to be *H. discodactylus* from animals he collected in the Rwandan and Kivu

montane forests. It appears that he did not visit the museum, but rather was sent material by Wermut who must have sent him only this one specimen of *H. discodactylus*, as there is no mention of any others. Furthermore, there is no mention of the *H. alticola* type specimens and it seems unlikely that they were included in his taxonomic review.

In 1972, Laurent published a second account on the explorations of Virunga National Park, listing 150 specimens of *H. discodactylus* (currently held at the Royal Museum for Central Africa, RMCA and the Museum of Comparative Zoology, MCZ) collected from 16 locations (Laurent 1972a). Less certain than he was 20 years earlier, he commented that although specimen collected from the north of the park (from Mt. Tashiaberimu, west of Kyavinyonge) resemble those from further south of the park, Nyungwe Forest and the Kahuzi region in DRC: "those of Rwenzori are quite different to warrant some doubts about the merits of the synonymy of *H. alticola* with *H. discodactylus* (Laurent 1972a). He commented that in the specimens from Rwenzori, the inner metatarsal tubercle was more developed, while the internarial space was narrower and the toe webbing reduced. Laurent (1972a) also noted that some females from Rwenzori were darker, something not observed in other populations. He insisted that these differences were not strong enough to resurrect *H. alticola*, but that they possibly indicated the beginning of speciation. In Ahl's original descriptions, snout shape and extent of webbing also differed between the two taxa, although he referred to *H. discodactylus* as being the darker animals and did not note any differences in tubercle form.

In 1975, Arne Schiøtz published the first call description for *H. alticola* and reported on his examination of the type material in Berlin, some of the RMCA material from Laurent's collections, and also his own material from Bwindi Impenetrable NP (Schiøtz 1975). He concluded that his own material fitted the description of *H. alticola* more closely than that of *H. discodactylus*, but that at least one of Laurent's specimens from Burunga (near Mokoto Lakes, just north of Goma, DRC, most likely referring to the Virunga Volcanoes in the Musanze-Goma region) and a specimen collected by Loveridge from Mubuku Valley (Eastern Rwenzori Mountains) in 1942 were the same as what he had collected. Schiøtz was unable to decide where to place the type specimen of *H. discodactylus*, suggesting it could be a conspecific of either *H. alticola* or *H. frontalis* Laurent, 1950, although this claim seems to be based solely on "the traces of colour left and in Ahl's description of the pattern" (Schiøtz

1975). Schiøtz (1975) is the first reference in which the type of *H. alticola* is listed as "lost?", although it is likely that already during Laurent's investigations, the type material was not available.

Since the studies of Laurent (1947, 1961, 1972a) and Schiøtz (1975), there have been no further detailed taxonomic assessments. Schiøtz's reversal in priority (1975, 1999) of *H. alticola* over *H. discodactylus* has not been accepted (Schiøtz & Drewes 2004; Frost 2013) but questions remain about their taxonomic status. In this study we document the rediscovery of the type material of *H. alticola* in the collection in Berlin, report on new records from outside its known distribution range and evaluate the validity of the proposed synonymy of *H. alticola* with *H. discodactylus*. We include molecular, acoustic and external morphological data from samples across the distribution range and investigate whether data supports the recognition of more than one taxon.

Material and methods

Generating molecular data

The mitochondrial 16S rRNA locus has been shown to perform well as a barcoding gene for amphibians at the species level (Vences *et al.* 2005). All available 16S sequences for *H. alticola* and *H. discodactylus* from GenBank (Benson *et al.* 2013) were downloaded and novel sequences were generated from freshly collected tissues. New samples were collected from Rwanda (Nyungwe Forest National Park and Gishwati Forest Reserve), DRC (Kahuzi-Biega National Park) and from Burundi (Bururi Forest Nature Reserve).

DNA was extracted following standard protocol from leg muscle tissue stored in >96% Ethanol, using Qiagen DNeasy tissue kit (Qiagen Inc, Valencia, CA, USA). Approximately 568bp fragments were amplified via Polymerase Chain Reaction (PCR), by two institutes using similar methods. The first used illustraTM puReTaq Ready-To-Go (GE healthcare, UK) PCR beads and forward and reverse primers published by Palumbi *et al.* (2002; 16SAL and 16SBH) with the following thermal cycling conditions: 5 minutes at 95°C, followed by 35 cycles of 1 minute at 95°C, 30s at 51°C and 90s at 72°C, followed by a final elongation phase of 7 minutes at 72°C. PCR products were visualized on 1% Agarose gels and successful amplifications were sent to Microsynth AG (Balgris, CH) for purification and sequencing. The

second institute used a slightly modified reverse primer (5'-CCGGTCTGAACTCAGATCACGT-3') and PCR products were purified using Highpure PCR Product Purification Kit (Roche Diagnostics, DE). Sequencing was performed with the DYEnamic ET Terminator Cycle Sequencing Premixkit (GE Healthcare, Munich, DE) for sequencing reactions run on a MegaBACE 1000) automated sequencer (GE Healthcare) at the laboratory of the Biogeography Department of Trier University. Complementing strands were sequenced and subsequently proofread using Codoncode Aligner v4.4.1 (Codoncode Cooperation, Centerville, MA, USA). All sequences generated de novo were deposited on GenBank and assigned accession numbers (Table 20).

Phylogenetic reconstruction

For the phylogenetic inference, *Afrixalus ulugurensis* (Barbour & Loveridge, 1928) was selected as an outgroup and a selection of *Hyperolius* species from the Albertine Rift and East Africa were sampled (*H. castaneus*, *H. lateralis* Laurent, 1940, *H. kivuensis* Ahl, 1931 and *H. cystocandicans* Richards & Schiøtz, 1977) to support the positioning of the *H.* cf. *discodactylus* and *H.* cf. *alticola* sequences in the phylogeny. The gene fragments were aligned using MUSCLE Alignment (Edgar 2004) with default settings in the bioinformatics tool suite Geneious Pro 5.6 (created by Biomatters, available from http://www.geneious.com). A Bayesian phylogenetic inference was carried out using MrBayes version 3.2.2 (Ronquist *et al.* 2012). Two runs, each with one cold Markov chain and three heated chains were executed for 5.5 million generations, sampling every 1000 generations. The chains were allowed to sample across the entire general time reversible substitution model space (lset nst=mixed, rates=gamma) to remove the need for a prior model testing. The parameter traces and interactions as well as the convergence of the two runs were inspected using Tracer version 1.4 (Rambaut & Drummond 2007) and 0.5 million generations were discarded as burn-in before generating a 50% majority-rule consensus tree from the posterior tree samples, adding all compatible groups to the tree (contype=allcompat). Intra- and inter-clade distances were calculated using the Species Delimitation plugin v1.04 for Geneious Pro (Masters *et al.* 2011).

Morphometric data

Specimens, including the type material of *H. discodactylus* and the rediscovered material of *H. alticola* were examined on-site or loaned from the Museum für Naturkunde, Berlin (ZMB)

Table 20. Vouchers represented in the molecular phylogeny with GenBank Accession numbers for all 16S rRNA sequences.

Voucher	Country	Locality	Accession number
RdS 835	Tanzania	Tegetero Village, Ulugurus Mts	KC179966
CAS:HERP:202372	Uganda	Kabale Dist.; pond E of Institute for Tropical Forest Conservation	FJ151059
ZFMK77611	Kenya	Mt. Kenya	GU443999
CAS:HERP:201986	Uganda	Uganda: Rukungiri Dist.: Bwindi Impenetrable National Park	AY603986
NMK A/3867/4			AY323919
SL471	Uganda	Bundibugyo	GU443979
NMK A/3925/1			AY323924
SL470	Uganda	Rwenzori Mts., Nyakalengija	GQ183573
SL472	Uganda	Rwenzori Mts., Nyakalengija	GQ183574
JMD775	Burundi	Bururi Forest Reserve	KF562042
Kaul_leBu (tadpole)	Burundi	Bururi Forest Reserve	KF562030
KAULdiBu (tadpole)	Burundi	Bururi Forest Reserve	KF562028
ZMB 78952	Burundi	Bururi Forest Reserve	KF562038
ZMB 78953	Burundi	Bururi Forest Reserve	KF562026
ZMB 78954	Burundi	Bururi Forest Reserve	KF562043
MTSN 6832	DRC	Kahuzi-Biega National Park; Bugulumiza	KF562039
MTSN 6835	DRC	Kahuzi-Biega National Park; Bugulumiza	KF562035
MTSN 6849	DRC	Kahuzi-Biega National Park; Bugulumiza	KF562045
MTSN 6850	DRC	Kahuzi-Biega National Park; Bugulumiza	KF562029
MTSN 6856	DRC	Kahuzi-Biega National Park; Bugulumiza	KF562031
MTSN 7209	Rwanda	Nyungwe Forest National Park; Nshili	KF562027
MTSN 7210	Rwanda	Nyungwe Forest National Park; Nshili	KF562032
MTSN 7211	Rwanda	Nyungwe Forest National Park; Nishili	KF562034
MTSN 7350	Rwanda	Nyungwe Forest National Park; Kamiranzovu	KF562024
MTSN 7364	Rwanda	Nyungwe Forest National Park; Mt. Bigugu	KF562023
MTSN 7365	Rwanda	Nyungwe Forest National Park; Mt. Bigugu	KF562046
MTSN 7366	Rwanda	Nyungwe Forest National Park; Mt. Bigugu	KF562044
MTSN 7367	Rwanda	Nyungwe Forest National Park; Mt. Bigugu	KF562033
MTSN 7369	Rwanda	Nyungwe Forest National Park; Mt. Bigugu	KF562037
ZMB 77536	Rwanda	Nyungwe Forest National Park; Waterfall	KF562036
ZMB 78947	Rwanda	Gishwati Forest Reserve	KF562025
ZMB 78948	Rwanda	Gishwati Forest Reserve	KF562040
ZMB 78950	Rwanda	Nyungwe Forest National Park; Rwasenkoko	KF562041
CAS:HERP:202047	Uganda	Bwindi Impenetrable Forest National Park, Munyaga River	AY603984
CAS:HERP:202074	Uganda	Bwindi Impenetrable Forest National Park, Munyaga River	DQ283225
SL453	Uganda	Rwenzori Mts.; Circuit Trail	GQ183572

and Museum for Comparative Zoology, Harvard (MCZ). Further collections from ZMB, MCZ, the California Academy of Science, California (CAS), Museo delle Scienze di Trento, Italy

(MUSE; specimen code MTSN) and from the collection of Stefan Lötters, University of Trier (SL) were loaned and measured. Specimens from the MCZ included individuals from the expeditions of G. F. de Witte and A. Loveridge, which had been included in previous revisions (Laurent 1947; Schiøtz 1975). In total, 63 adult specimens (46 males and 17 females) from eight localities were included in the morphological analysis (Table S[=Appendix] 1).

Fifteen external morphological measurements were taken using dial callipers, to the nearest 0.1 mm with the aid of a Leica MZ8 stereo microscope (Leica Microsystems GmbH, Wetzlar, Germany): snout-vent length (SVL), measured from snout tip to posterior margin of vent; snout-urostyle length (SUL), measured from snout tip to posterior margin of the urostyle; width of the internarial space (INS); eyelid width (EW); canthus length (CL), measured from the centre of the nostril to the anterior corner of the eye; width of the interorbital space (IOS), measured at the narrowest point; head width (HW), measured at the widest point; 1st toe length (1TL); 2nd toe length (2TL); 3rd toe length (3TL); 4th toe length (4TL), 5th toe length (5TL), all measurements including the metatarsus, from the proximal end of the inner metatarsal tubercle, to the distal end of the phalanges, including toe discs; femur length (FL); tibia length (TibL) and tarsal length (TarL). SVL was measured to allow for comparison to body size references in the literature; however it was not included in the morphometric analysis as SUL is a more objective measurement of body size for preserved specimen. The specimens were sexed by the presence or absence of a vocal sac and examination of the gonads through lateral incisions. Toe webbing formula was noted to the closest quarter of a phalange following Savage & Heyer (1997). The median and interquartile range was then graphically displayed. Two investigators measured the specimens, only one of which assessed webbing.

Morphometric analysis

To visualize the morphological variation all fourteen morphometric variables (Table S1) were log transformed to better conform to normality and subjected to a rigid rotation via a Principal Component Analysis (PCA) using the prcomp function in R (R core team 2013). A Linear Discriminant Analysis (LDA) was carried out with the R packages mass (Venables & Ripley 2002) and vegan v.2.0-8 (Oksanen *et al.* 2013) using the two recovered phylogenetic clades (*H.* cf. *alticola* and *H.* cf. *discodactylus*; see results) as an a priori grouping variable. To account for sexual dimorphism, yet retain comparable axes, the groups were divided into

male and female classes, resulting in four groups. Posterior probabilities were then calculated for each point to assess whether the a priori clades can accurately separate the specimens based on morphology. Samples from the same locality were assumed to be from the same genetic lineage and to test the robustness of this method explicitly, the two genetically different samples from Gishwati FR (see results) were excluded from the discrimination computations. Using the "predict" function in R, they were then assigned to one of the four classes a posteriori. If the underlying genetic differences also reflect morphological differences recoverable by the LDA, the two samples should be placed in differing classes. Similarly, the "predict" function was used to assign the syntype of *H. discodactylus* collected from west of Lake Edward (MCZ A-17634) to one of the two morphotypes, as no genetic material was available from this region. 95% confidence intervals were drawn on plots with the R package ellipse (Murdoch & Chow 1996). To provide biologically meaningful information about which morphological traits best separate the two genetic groups, each morphometric variable was subjected to an Analysis of Covariance (ANCOVA) with sex and morphotype (*H.* cf. *alticola* or *H.* cf. *discodactylus* as determined by the LDA) as independent variables and SUL as a covariate to accommodate allometry. Two particularly small individuals, CAS 180449 and ZMB 78954 (Table S1) are likely to be subadults and were therefore not included in the analyses.

To meet the underlying assumptions of the statistical tests used, homogeneity of within-group covariance and normality of data were checked and adhered to. To inspect whether two investigators measuring specimens created artificial patterns in the data, a Multivariate Analysis of Covariance (MANCOVA) was constructed with PC scores for all morphometric data, excluding SUL as a multivariate dependent variable, against SUL, sex, morphotype and "investigator" as independent co-variables.

Acoustic analysis

Advertisement calls were recorded in Gishwati Forest and Nyungwe Forest, Rwanda, and in Bururi, Burundi, with a Sony PCM–D50 Linear PCM Recorder with stereo microphones (Sony Deutschland GmbH, Cologne) by JMD; and in Kahuzi-Biega National Park, DRC, with an Olympus LS-10 PCM digital stereo audio recorder by MM. Ambient temperature during the recordings was between 12.4 and 18.3 °C in Nyungwe NP, 14.6 and 15.6 °C in Gishwati, 9.2 and 14.4 °C in Kahuzi-Biega, and 15.4 and 18.7 °C in Bururi. Call recordings were analyzed

using Adobe Audition 1.5 software. Stereo recordings were converted to mono at a sampling rate of 44.1 kHz and resolution of 16 bits. Temporal data were obtained from oscillograms and frequency information was obtained from sound spectrograms using Fast Fourier Transformation (1024 point Blackman window). Definitions of acoustic parameters follow Duellman (1970) and Dehling & Matsui (2013).

Geographic range

Locality information was downloaded from the Global Biodiversity Information Facility (GBIF; http://www.gbif.org) and complemented with unlisted records from literature and online sources as well as the novel records of the authors (Table S7). Anecdotal records were geo-referenced using Google Earth v.7.1.1 (Google Inc., Mountain View, CA), respecting verbatim altitude references wherever available. Type localities were estimated as accurately as possible from sites depicted on maps of the collection expeditions by R. Grauer under Herzog Adolf Friedrich (Schubotz 1913). A map of all known localities overlaid with the IUCN red list expert range map (Schiøtz & Drewes 2004) was produced using ArcGIS v10.1 (ESRI, Redlands, CA, USA).

Results

Rediscovery of the syntypes of* H. alticola *Ahl, 1931

In the late 1970s (after 1975, at which time Schiøtz was still unable to locate the specimens) the type material of *H. alticola* was catalogued by an unknown individual. To our knowledge, this rediscovery was not made public. At present, two female specimens fitting the description and diagram by Ahl (1931b), with labels floating freely inside the jar are located in the ZMB collection. One specimen is considerably larger and matches the snout-vent length provided in the original description and is hereby designated as the lectotype with the older collection number ZMB 39008 (Figure 44a&b). The smaller individual is assigned the more recent number ZMB 74944 and is designated as a paralectotype (Figure 44c&d). Based on the map of the German Central African Expedition (Schubotz 1913), the most likely locality for both specimens and therefore the type locality of *H. alticola* is Butago, east of the city of Beni, heading towards Stanley Peak, on the western slope of the Rwenzori Mountains in DRC (Figure 45).

Only one specimen of *H. discodactylus* was found in Berlin, a male from Nyungwe Forest, Rwanda, which was designated as the lectotype by Laurent (1961) and given the number ZMB 36089 (Figure 44e&f). Therefore, Nyungwe Forest (most likely Rwasenkoko [Uwasenkoko]) is the restricted type locality for *H. discodactylus* (Figure 45). Of the original seven specimens mentioned by Ahl, only one more could be located, the specimen on loan in Harvard, MCZ A-17634, from "west of Lake Albert-Edward" which has paralectotype status (Figure 44g&h). The Grauer expedition made three stops on the western banks of Lake Edward; in Mokokoma, Angi and Amakoma (Schubotz 1913; Figure 45). The adjacent mountains along the shoreline reach 2500 m a.s.l., now within the Tayna and Kisimba-Ikobo Community Reserves, which is the likely place of origin of this paralectotype. The whereabouts of the remaining five paralectotypes is still unknown. Enquiries at natural history museums and institutes were made including the American Museum of Natural History (AMNH), the Field Museum of Natural History (FMNH) and the British Natural History Museum (NHM) but did not result in the discovery of additional specimens.

Phylogenetic reconstruction

Bayesian Inference was able to recover well-supported geographic clades with the exception of samples from Gishwati FR, which belonged to two separate clades (Figure 46). Although genetic distances roughly correspond to geographic distances, animals from Kahuzi-Biega NP appear to be more closely related to those from Bwindi Impenetrable NP and Rwenzori than to those from the geographically adjacent Nyungwe Forest NP. Nonetheless, two major clades dividing the samples into a northern and a southern group are recovered, with intra-clade patristic distances of 0.118 and 0.110 respectively, compared to an inter-clade

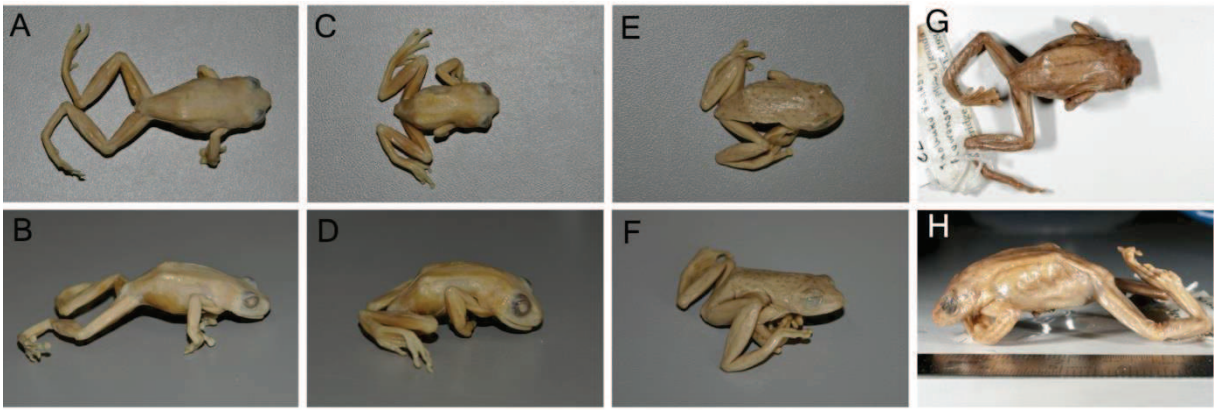

Figure 44. Photographs of type material of *H. alticola* (a–b: ZMB 39008; c–d: ZMB 74944) and *H. discodactylus* (e–f: ZMB 36089; g–h: MCZ A-176634).

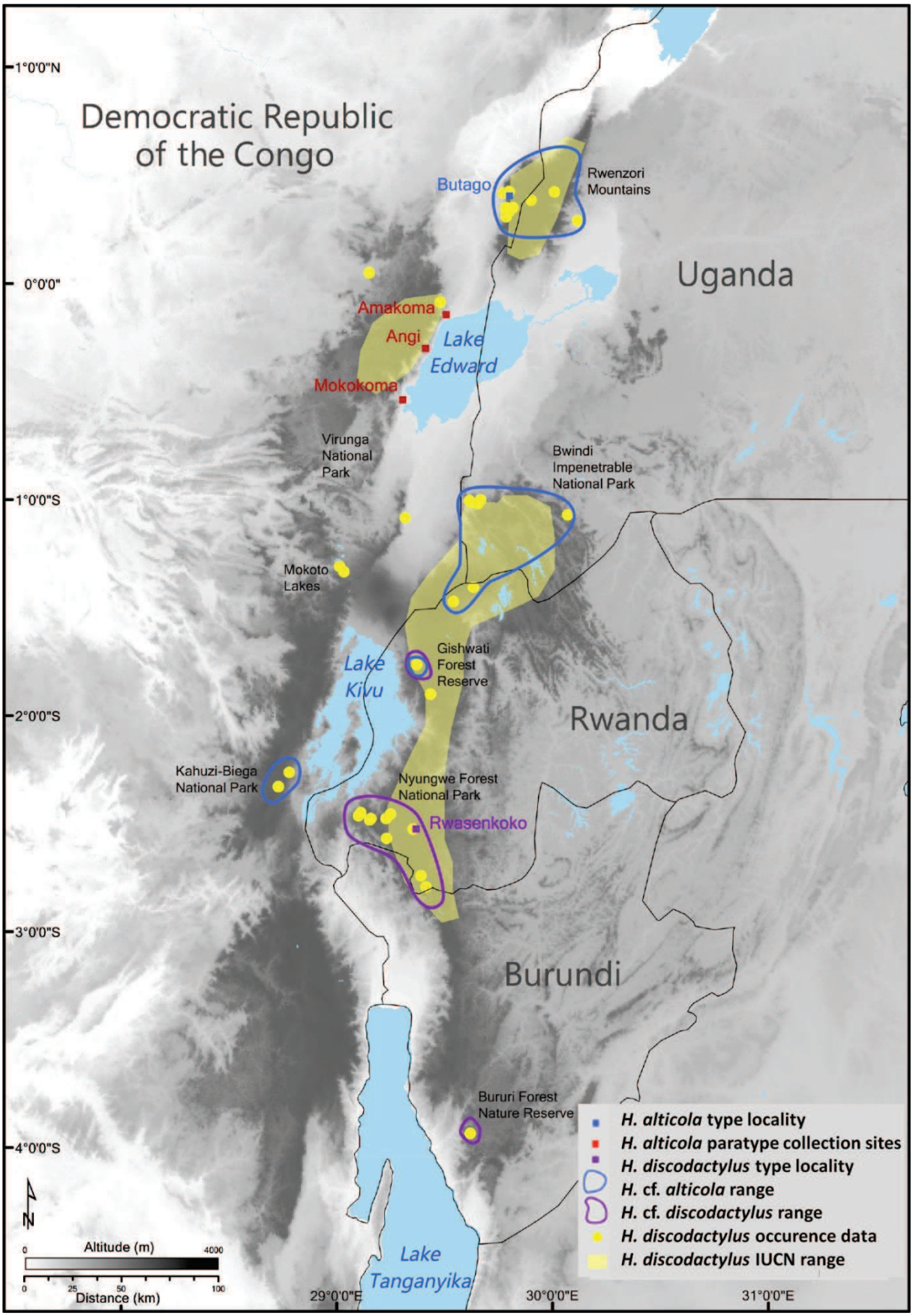

Figure 45. Distribution of *H. discodactylus* along the Albertine Rift.

distance of 0.305. The pairwise base difference between the two clade has a median of 1.9%, reaching a maximum of 3% between the samples SL 453 and MTSN 7209-7211, and although these are also some of the geographically most widely separated specimens, samples from Nyungwe Forest NP and neighbouring Kahuzi-Biega NP nonetheless have differences of up to 1.9% and the two samples from Gishwati FR, presenting itself as a geographic contact zone between the two ranges, have accumulated 1.9% base-pair differences. The two clades represent the different type localities of *H. alticola* (Rwenzori Mountains) and *H. discodactylus* (Nyungwe Forest) and are therefore referred to as *H.* cf. *alticola* and *H.* cf. *discodactylus* respectively for subsequent morphological analyses.

Morphometric analysis

Means and standard deviations of all measured specimens per locality are given in Table 21, and representative photos of specimens in situ from different regions are provided in Figure 46a–g. A table of all individual measurements including the type series is provided in the supplementary materials (Table S1). The PCA of all morphometric variables shows a separation of males and females along the first PC axis with the second axis providing little extra separation of the data (Figure 47), except marginal clustering by locality. Geographically adjacent animals from Nyungwe Forest NP and Kahuzi-Biega NP cluster together, separated from Bwindi Impenetrable NP and Rwenzori Mountains animals by PC1. There is no single predominant loading variable accounting for the dispersion of data along the first axis, indicative of strong collinearity of variables. Interorbital space is the main loading variable for PC2 (Table S2), which separates Bwindi Impenetrable NP from the rest. The first two principal components account for 73.6% of the variance observed in the data and only by component eight is more than 95% of the cumulative variance accounted for.

The sample from Ituri forest (CAS 196153) shows strong deviation from the rest of the data cloud when plotting components three against four (Figure S1), whose loadings are dominated by eyelid and canthus measurements respectively. Ituri Forest is located west of the Albertine Rift, considerably outside the known distribution range of H. discodactylus and the sample was collected at approx. 650 m below the lower altitude bound of this species. It is therefore possible that this specimen belongs to a different species and was removed from further analyses.

Table 21. Means (±SD) of morphometric measurements (in mm) for each locality for snout-vent length (SVL), snout-urostyle length (SUL), internarial space (INS), eyelid width (EW), canthus length (CL), interorbital spaces (IOS), head width (HW), 1st to 5th toe lengths (1-5TL), femur length (FL), tibia length (TibL) and tarsus length (TarL).

			SVL	SUL	INS	EW	CL	IOS	HW	1TL	2TL	3TL	4TL	5TL	FL	TibL	TarL
Bwindi Impenetrable National Park	Females	*N=6*	31.87 (±1.63)	31.50 (±1.42)	3.00 (±0.43)	2.45 (±0.22)	3.22 (±0.35)	4.45 (±0.40)	11.78 (±0.57)	5.43 (±0.45)	8.28 (±0.92)	12.08 (±0.68)	15.57 (±0.66)	12.60 (±0.70)	15.77 (±0.87)	16.32 (±0.80)	9.20 (±1.08)
	Males	*N=22*	28.67 (±1.37)	28.23 (±1.36)	2.56 (±0.23)	2.17 (±0.24)	2.70 (±0.23)	3.94 (±0.30)	10.19 (±0.64)	5.00 (±0.46)	7.28 (±0.73)	10.82 (±0.84)	13.58 (±0.99)	11.45 (±0.90)	14.15 (±0.75)	14.41 (±0.75)	7.97 (±0.47)
Ituri Forest	Females	*N=1*	31.50	31.80	2.40	1.90	3.60	4.20	10.50	5.50	6.30	10.50	12.80	10.50	15.60	16.30	10.20
	Males	*N=0*															
Kahuzi-Biega National Park	Females	*N=2*	35.65 (±1.48)	35.05 (±2.05)	3.25 (±0.21)	2.60 (±0.00)	3.25 (±0.21)	5.50 (±0.28)	12.60 (±0.28)	6.40 (±0.28)	9.20 (±0.00)	12.55 (±0.07)	16.05 (±0.07)	13.75 (±0.64)	17.50 (±0.42)	19.10 (±0.42)	9.35 (±0.07)
	Males	*N=4*	32.08 (±1.53)	32.05 (±1.75)	2.75 (±0.33)	2.38 (±0.30)	2.90 (±0.14)	5.15 (±0.25)	10.73 (±0.36)	5.55 (±0.34)	8.30 (±0.18)	11.40 (±0.42)	15.03 (±0.54)	12.08 (±0.45)	15.80 (±0.90)	16.68 (±0.41)	8.93 (±0.33)
Nyungwe Forest National Park	Females	*N=2*	34.50 (±0.28)	33.35 (±0.92)	3.30 (±0.14)	2.40 (±0.14)	3.30 (±0.14)	5.20 (±0.28)	12.55 (±0.64)	6.55 (±0.78)	9.10 (±1.56)	12.95 (±0.64)	16.10 (±0.71)	12.60 (±0.14)	16.65 (±1.20)	17.75 (±1.34)	9.30 (±0.14)
	Males	*N=9*	31.73 (±1.79)	30.96 (±1.92)	2.86 (±0.22)	2.30 (±0.21)	2.92 (±0.20)	4.99 (±0.45)	11.27 (±0.48)	5.39 (±0.56)	8.02 (±0.49)	11.51 (±0.68)	14.57 (±1.14)	11.82 (±0.86)	15.21 (±1.01)	15.90 (±0.84)	8.63 (±0.54)
Rwenzori Mountains	Females	*N=5*	35.12 (±2.93)	34.64 (±2.49)	3.30 (±0.24)	2.64 (±0.26)	3.50 (±0.33)	5.38 (±0.19)	12.22 (±0.49)	6.16 (±0.52)	8.62 (±0.48)	12.22 (±0.51)	16.48 (±0.86)	13.20 (±0.87)	16.44 (±0.68)	17.00 (±0.93)	9.88 (±0.45)
	Males	*N=6*	29.67 (±0.63)	28.50 (±0.41)	2.80 (±0.18)	2.08 (±0.19)	2.73 (±0.21)	4.78 (±0.19)	9.73 (±0.15)	4.82 (±0.23)	7.22 (±0.43)	10.52 (±0.27)	13.23 (±0.35)	10.85 (±0.36)	13.78 (±0.56)	14.62 (±0.39)	7.92 (±0.35)
Gishwati Forest Reserve	Females	*N=1*	38.40	38.80	3.30	2.70	3.50	6.40	13.80	6.30	10.10	15.40	18.80	15.60	18.30	19.40	10.00
	Males	*N=1*	33.60	32.60	2.70	2.40	2.90	5.00	11.30	5.30	7.80	11.70	15.60	11.70	16.30	18.00	9.80
Bururi Forest Nature Reserve	Females	*N=0*															
	Males	*N=3*	30.27 (±0.38)	29.00 (±0.20)	2.83 (±0.06)	2.23 (±0.15)	2.60 (±0.10)	4.73 (±0.12)	10.53 (±0.58)	4.73 (±0.47)	7.30 (±0.10)	11.13 (±0.46)	13.37 (±0.29)	11.17 (±0.42)	13.87 (±0.55)	14.53 (±0.38)	7.70 (±0.36)
West of Lake Edward	Females	*N=0*															
	males	*N=1*	32.20	32.1	2.80	2.2	2.70	4.9	10.20	5	6.90	11.4	14.50	12	15.70	16.5	10.40

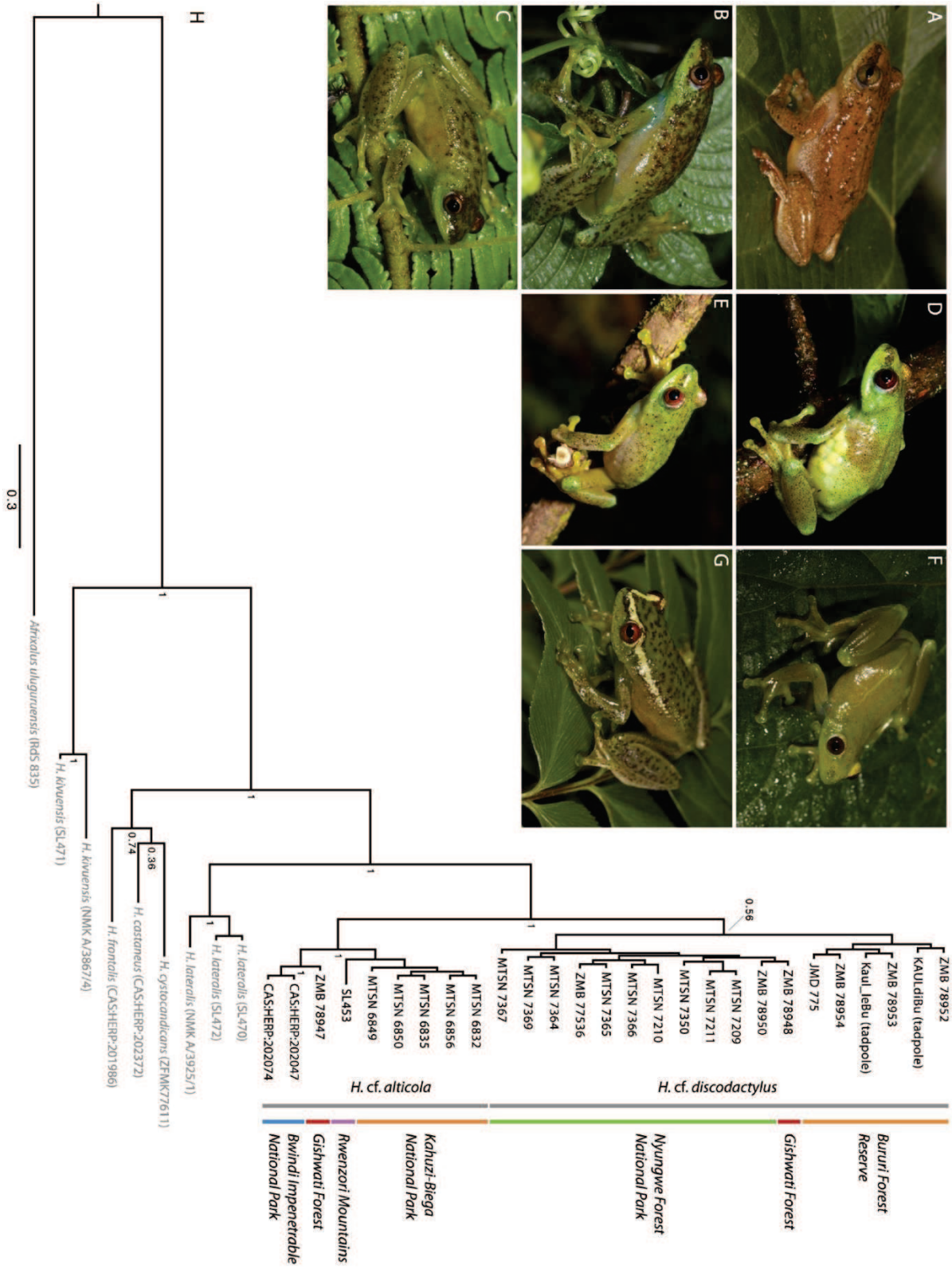

Figure 46. Photographs of *H. discodactylus* in situ from different localities. (A) Rwenzori Mountains, female; (B) Gishwati FR, male; (C) Bururi Forest NR, male; (D) Nyungwe Forest NP, female; (E) Nyungwe Forest NP, male; (F) Kahuzi-Biega NP, female; (G) Kahuzi-Biega NP, male; and (H) consensus tree of phylogenetic reconstruction using Bayesian inference on 16S mtDNA sequences. Node labels are posterior probabilities.

The Linear Discriminant Analysis (Figure 48) separated males and females of the same genetic group by LD1 and the two genetic groups by LD2. Six of the 58 specimens included in the analyses showed discrepancies between the prior and predicted classifications (Tables S3 & S4) with one being placed in the wrong gender category. Nonetheless, four of these six samples fall within the 95% confidence ellipse of their a priori group and the method correctly placed the two Gishwati FR samples into their respective genetic clades (ZMB 78948, *H.* cf. *discodactylus* with 65.7% posterior probability; ZMB 78947, *H.* cf. *alticola* with a 66.2% posterior probability; Table S3). The syntype of *H. discodactylus* (MCZ A-176634) was classified as belonging to the *H. alticola* clade (posterior probability of 99.5%; Table S3).

The MANCOVA on PC scores showed no significant interaction between the independent variables and no effect of having two investigators carrying out the morphological measurements (approx. $F= 1.831$, $p= 0.075$; Table S5). Strong support for allometry in morphology (approx. $F=39.428$, $p<0.001$; Table S5) was recovered as well as evidence for both sexual dimorphism and variation in morphology between the *H.* cf. *alticola* and *H.* cf. *discodactylus* (sex: $F=3.9701$, $p<0.001$, predicted groups: $F=2.956$, $p<0.05$; Table S5). Subsequent ANCOVAs revealed that SUL, INS, CL, HW, 3TL, 4TL and 5TL showed sexual dimorphism and SUL, CL, IOS and HW differed significantly between *H.* cf. *alticola* and *H.* cf. *discodactylus* (Table S6; Figure 49), with weak, but significant interaction terms for INS and HW between sex and morphotype. Females are larger, have longer canthi, wider heads, and longer 3rd and 4th toes, but have relatively narrower internarial spaces and shorter 5th toes than males (Figure 49). *H.* cf. *discodactylus* males are slightly larger, have shorter canthi, wider interorbital spaces and wider heads than males of *H.* cf. *alticola* (Figure 49). The dataset contains only two records for female *H.* cf. *discodactylus* and thus no meaningful statistical comparisons can be made.

Webbing is largely conserved both between morphotypes and sexes, with *H.* cf. *discodactylus* males and females showing slightly reduced webbing on the fourth toe compared to their *H.* cf. *alticola* counterparts (Figure 50).

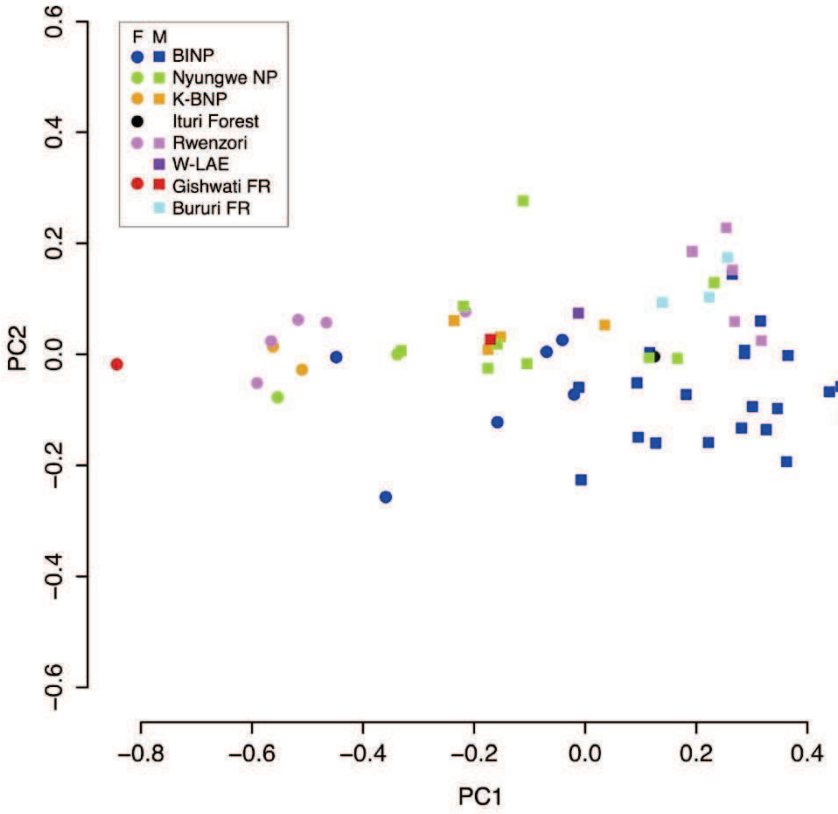

Figure 47. Biplot of the first two Principal Components of a PCA on all fourteen log transformed morphometric variables for *H. discodactylus*.

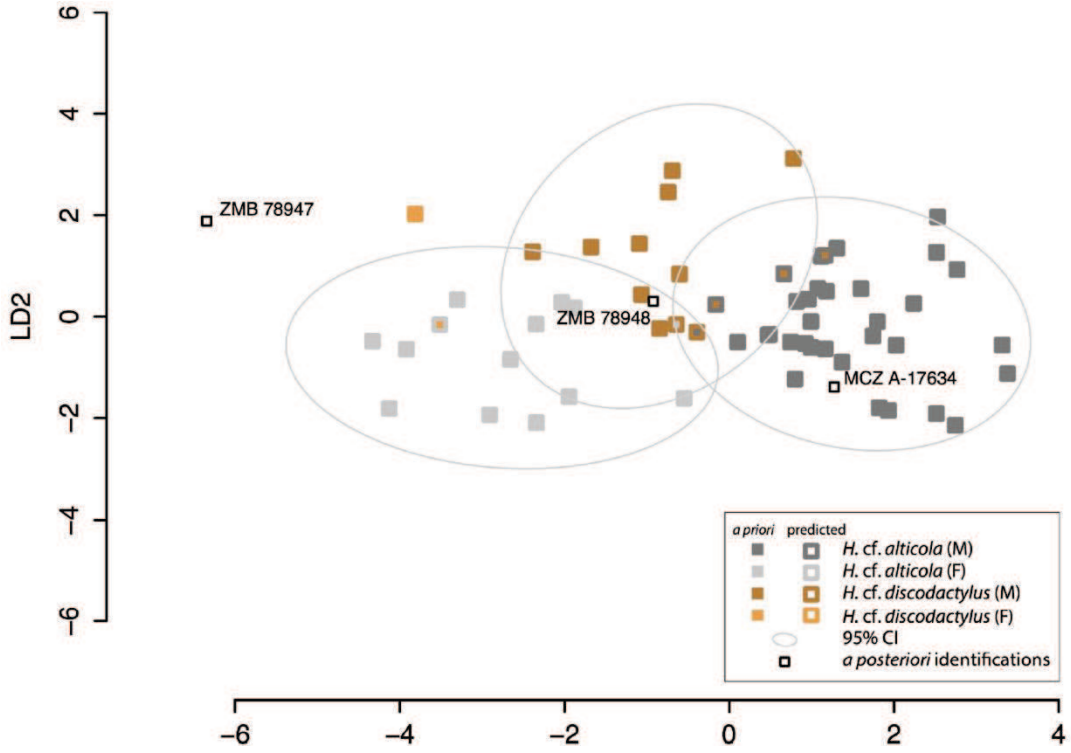

Figure 48. LDA plot showing the a priori and predicted classifications of male and female *H.* cf. *alticola* and *H.* cf. *discodactylus* and a posteriori placements of the samples from Gishwati (ZMB 78947 and 78948) and the paralectotype from west of Lake Edward (MCZ A-17634).

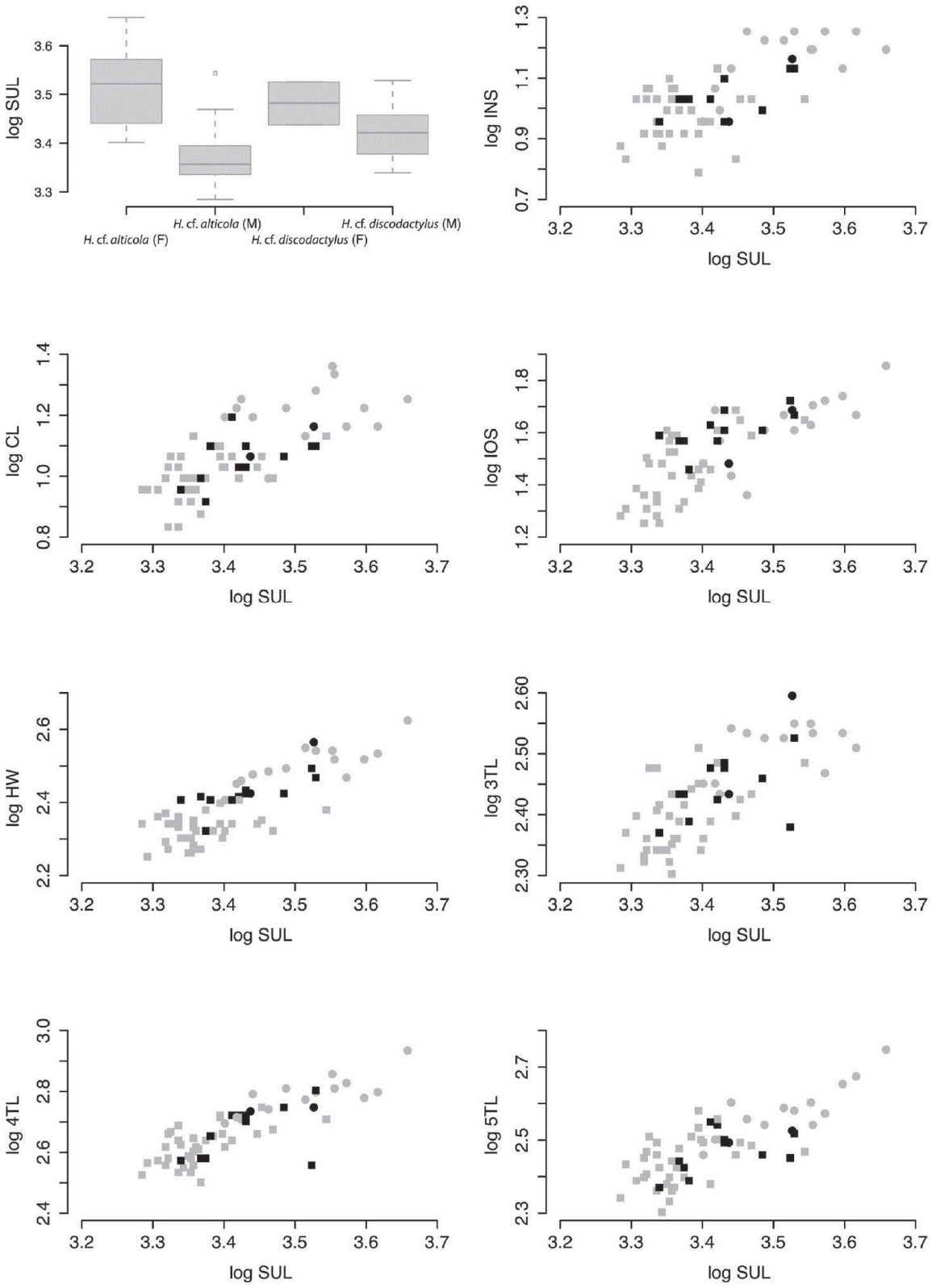

Figure 49. Scatter plots of selected morphological variables (internarial space [INS], canthus length [CL], interorbital space [IOS], head width [HW], 3rd toe length [3TL], 4th toe length [4TL] and 5th toe length [5TL]) against snout-urostyle length (SUL) that show statistically significant differences between either sex or morphotypes. Circles represent females, squares represent males and grey and black represent the two morphotypes, *H.* cf. *alticola* and *H.* cf. *discodactylus* respectively.

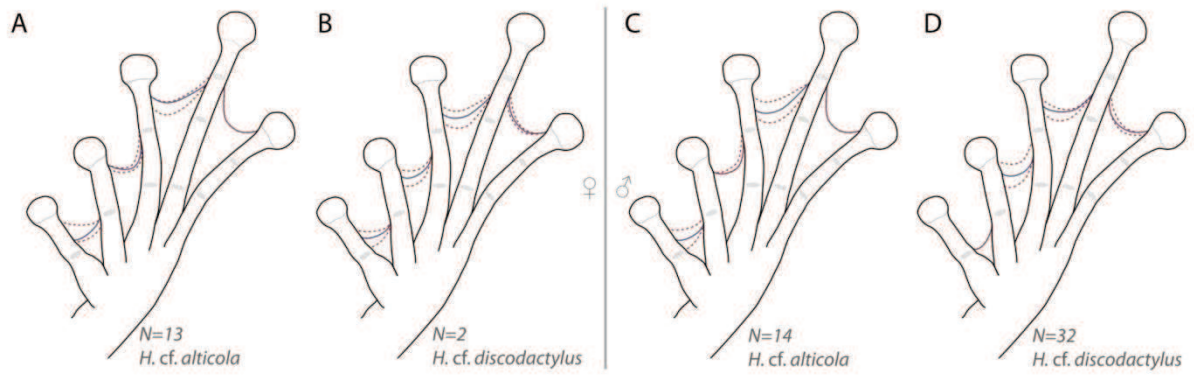

Figure 50. Schematic drawing of the webbing of left hind limb for (A) *H.* cf. *alticola* females, (B) *H.* cf. *discodactylus* females, (C) *H.* cf. *alticola* males; and (D) *H.* cf. *discodactylus* males. Blue lines represent the median number of free phalanges and dashed red lines represent the interquartile range. Numbers of individuals per morphotype are given.

Advertisement call

Specimens were recorded at night while calling from vegetation between 1 and 3 m above the ground at the edge of forest swamps and along small streams in the forest. Calls from Gishwati FR, Nyungwe NP, and Kahuzi-Biega NP were very similar to each other. The advertisement call recorded at these locations consisted of a single pulse group (note), which was composed of 19–43 pulses and had a duration of 167–362 ms (Table 22). Pulse repetition was highest at the beginning of the note and declined towards the end (Table 22). Only in few calls, pulse repetition rate was nearly constant from beginning to end. Amplitude modulation was prominent. Relative amplitude rose from the beginning to the middle of the call and declined towards the end. Frequency modulation was distinct. Energy maximum was at 1950–2350 Hz at the beginning of the call, reached maximum of 2350–2750 Hz at about three-fourths the call length, and slightly declined again towards the end. Frequency at maximum was 150–550 Hz higher than at the beginning of the call (Table 22). Prominent harmonics were at about 4300–5000 Hz, 6700–7400 Hz, and 8800–9500 Hz (Figure 51). Usually, calls were emitted in short series of up to six calls (Table 22; Figure 51) during which they were repeated at a rate of 0.6–1.2 calls per second (Table 22). Advertisement calls recorded at Bururi differed from those recorded elsewhere in being much shorter (82–98 ms) and consisting of a lower number of pulses (14–17), a higher dominant frequency, and a higher pulse repetition rate; call series were much longer and calls were repeated at a higher rate (Table 22; Figure 51).

Table 22. Properties of advertisement calls of *H. discodactylus* recorded at four different geographical regions. Values are given either as range or as median followed by range (in parentheses). N refers to the number of individuals recorded, calls to the number of analyzed calls.

Region	N	Calls	Note length [ms]	No. of pulses	Pulse repetition rate (beginning; end of note) [Hz]	Dominant frequency Beginning of note [Hz]
Gishwati Forest	3	15	206 (167–257)	29 (19–41)	125–250; 71–200	1950–2350
Nyungwe NP	12	71	263 (173–362)	37 (23–50)	142–250; 58–125	2000–2350
Kahuzi-Biega NP	3	34	240 (204–305)	28 (23–43)	111–220; 58–142	2000–2300
Bururi	5	51	92 (82–98)	16 (14–17)	250–285; 125–142	2550–2700

Region	N	Dominant frequency end of note [Hz]	Frequency modulation [Hz]	Calls per series	Call repetition rate [Hz]
Gishwati Forest	3	2500–2550	150–550	3 (1–6)	1.1 (0.9–1.2)
Nyungwe NP	12	2300–2700	150–500	3 (1–6)	0.8 (0.6–0.9)
Kahuzi-Biega NP	3	2350–2750	200–550	3 (1–3)	0.9 (0.6–1.0)
Bururi	5	2900–3000	250–350	8 (1–19)	2 (1.7–2.2)

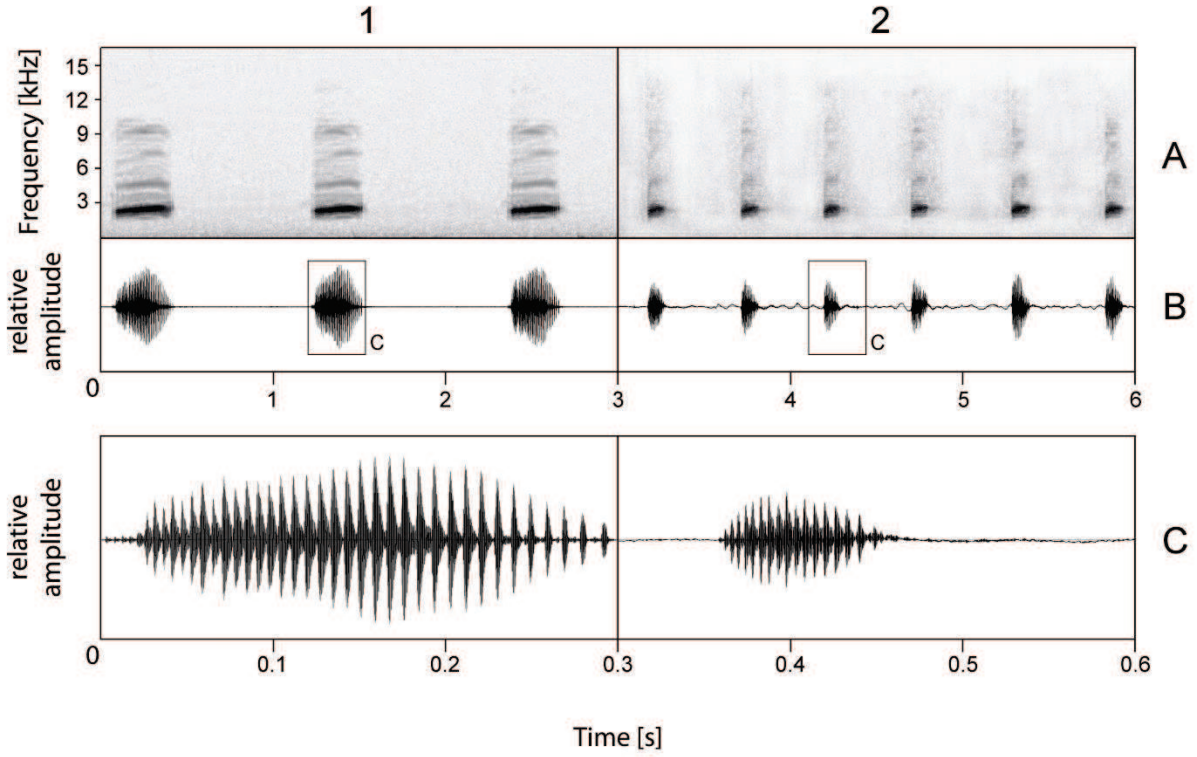

Figure 51. (A) Audiospectrograms and (B) corresponding oscillograms of call series and (C) oscillograms of single advertisement calls of *H. discodactylus* from Nyungwe NP, Rwanda (1; left) and from Bururi, Burundi (2; right).

Geographic range

When combining all historic and recent records listed under *H. discodactylus* and *H. alticola*, animals are restricted to montane areas ranging from a northern limit at the Rwenzori Mountains on the Uganda-DRC border, down the full length of the Virunga NP, DRC, to its

southwestern limits in Kahuzi-Biega NP, DRC. The range extends further south on the east side of Lake Kivu, from Bwindi Impenetrable NP in southwestern Uganda, through the Virunga Mountains in Uganda, DRC and Rwanda, the Gishwati FR and Nyungwe Forests NP in western Rwanda, reaching its ultimate southern limit in Bururi Forest NR in southern Burundi (Figure 51; Table S7). There is little suitable habitat left between Gishwati FR and Nyungwe Forest NP and between Nyungwe Forest NP and Bururi Forest NR and these are therefore likely to be fragmented populations rather than representatives of a continuous range (Laurent 1983a).

Compared to the range published on the IUCN red list website (Schiøtz & Drewes, 2004), collection records suggest a wider distribution across the Virunga National Park with central records from Mokoto Lakes and southern records from Kahuzi-Biega NP (Laurent 1950b). The known range can be extended considerably further south, based on new locality records from Bururi Forest NR in southern Burundi (Figure 51).

Discussion

Previous authors have investigated the validity of *H. discodactylus* and *H. alticola* as independent taxonomic units (Laurent 1972a; Schiøtz 1975), however this is the first study to include molecular data, statistical morphometric and acoustic analyses and most importantly, the type material of *H. alticola*.

Assessing the taxonomic validity of *H. discodactylus* and *H. alticola* based solely on type material is extremely problematic. Seven specimens were used to describe *H. discodactylus*, but only two could be located, and both are males. The type material of *H. alticola* consists only of two females. It is unclear, but unlikely that the missing five *H. discodactylus* paratypes were females given the distinctiveness of females, which should have been apparent to Ahl. It is therefore reasonable to assume that the two taxa were described based only on male specimens of *H. discodactylus* and female specimens of *H. alticola*. Given the considerable morphological variation exhibited between males and females, comparisons between male *H. discodactylus* and female *H. alticola* type material have to take into consideration this dimorphism, which seemingly Ahl did not.

In Ahl's (1931a) paper it can be discerned that three morphometric features differ between

H. discodactylus and *H. alticola*: Body size, interorbital space and tibia length. The morphometric analyses carried out in our study show that differences in two of these, body size and interorbital space, are largely sexually dimorphic and there is no evidence to suggest that tibia length varies significantly. Other more qualitative differences listed by Ahl include the visibility of the tympanum, toe webbing, dorsum colouration and granulation of the venter (Ahl 1931b). Colouration is not an accurate diagnostic feature for many amphibians and hyperoliids in general (Schiøtz 1999), especially when dealing with preserved material (Laurent 1950b). The remaining traits are also highly susceptible to preservation artefacts and if anything, the *H. discodactylus* morphotype has more reduced webbing, contrary to Ahl's notes. Based on Ahl's descriptions there is thus little support for a clear division of these animals into separate species.

The reconstructed phylogeny based on 16S rRNA recovers two well supported clades with the maximum interclade base-pair difference (3%) being similar to interspecific distances of other *Hyperolius* species groups (Schick *et al.* 2010; Conradie *et al.* 2012; Dehling 2012a). Furthermore, there is evidence that at least at one locality (Gishwati FR) these two lineages occur in sympatry. The two clades roughly correspond to animals from i) a northern distribution from the Rwenzori Mountains in Uganda, through Bwindi Impenetrable NP and Gishwati FR on the eastern side of Lake Kivu, to Kahuzi-Biega NP on the western side of the lake in DRC (i.e. Ahl's *H. alticola*) and ii) a southern distribution, starting from Gishwati FR in northwestern Rwanda, along the eastern side of Lake Kivu down through in Nyungwe Forest NP and in southern Burundi (i.e. Ahl's *H. discodactylus*). The LDA on external morphometrics lends morphological support to this grouping, with close to 90% showing strong affinities to their assigned group.

The proposed split based on the molecular data is not reflected in the acoustic analysis. The advertisement calls recorded at Gishwati Forest, Nyungwe NP, and Kahuzi-Biega NP were largely identical and match the description of calls recorded in Uganda (Schiøtz 1999; Channing & Howell 2006). This corroborates our findings that these populations likely represent only a single species, as recent studies on other species of *Hyperolius* have shown that the advertisement calls usually differs distinctly even between closely related species (Conradie *et al.* 2012, Dehling 2012a, Channing *et al*. 2013). The call from the southernmost locality at Bururi differed considerably from the "typical" advertisement call. The specimens,

however, do not differ in morphology and only slightly genetically from specimens from Nyungwe (p distance 0.4 %). Acoustic differences noted between populations therefore deserve further research and might be of taxonomic significance.

Data in favour of delimiting two species therefore exists, but no dataset provides conclusive evidence, which explains why previous researchers have struggled to make a definitive decision on the matter. The molecular divergences between some of the individuals are marginal (minimum inter-clade base-pair differences of 1.6%) and whether the recovered morphological differences are enough to stretch beyond population-level variances cannot be answered unequivocally. Interpreting morphological variation is further complicated by the strong effect of sexual dimorphism, which accounts for most of the variation. Furthermore, measurements were taken from material of vastly differing ages (collections from 1908 to 2013), which can also cause a range of error that hampers determining minimal morphological differences among populations. The paralectotype of *H. discodactylus* collected from west of Lake Edward (MCZ A-176634) presents a further discordance. In morphology, it best resembles the southern *H.* cf. *discodactylus* clade and Ahl was therefore correct in recognizing these similarities. However, geographically, it should be affiliated with the *H.* cf. *alticola* clade. The acoustic data is similar, with the greatest variances in advertisement calls being observed within the *H.* cf. *discodactylus* clade, not between *H.* cf. *discodactylus* and *H.* cf. *alticola*. The inconsistencies in genetics, morphology and acoustics in general, therefore point towards an incomplete separation of lineages, a pattern in favour of population-level variances. In this scenario, we have opted for taxonomic stability and therefore support Laurent's (1947) synonymy of *H. alticola* with *H. discodactylus*.

Further research on montane hyperoliids of the Albertine Rift is clearly necessary. In addition to understanding species diversity, the area is of high biological importance (Plumptre *et al.* 2007). Similar to patterns observed in other montane taxa (Tolley *et al.* 2010; Loader *et al.* 2013), areas of lowland are potential barriers to gene flow, with individuals of *H. discodactylus* from adjacent Nyungwe Forest NP and Kahuzi-Biega NP showing unexpectedly high levels of genetic divergence. Due to its fragmented range with a distribution across most of the Albertine Rift, this species would serve as an interesting model for testing biogeographic hypotheses of the evolutionary history of the fauna of the mountain chains.

Conclusion

Hyperolius in general show strong sexual dimorphism, a characteristic also present in the studied species. *H. discodactylus* and *H. alticola* were most likely described based only on preserved males of the former and females of the latter. The diagnostic features provided by Ahl (1931a, 1931b) are attributable to sexual dimorphism, population variation and potentially preservation age of material. The biological relevance of the few morphometric, acoustic and molecular differences recovered in this study are not unequivocal and we therefore concur with previous authors (Laurent 1947; Schiøtz 1975), that there is little evidence to support the recognition of two separate species. Although Schiøtz (1975) argues that the description of *H. alticola* correspond better to what he understands to be the synonymised *H. discodactylus*, we do not find support for this claim and stand by the argument of page priority for *H. discodactylus* as proposed by Frost *et al.* (2006). We also extend the known range of this species considerably to the south, with new records from Bururi Forest Nature Reserve, Burundi.

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Summary and prospects

In this thesis I investigated the diversity within amphibian communities in cultivated areas in Rwanda and within two selected, taxonomically challenging groups, the genera *Ptychadena* and *Hyperolius*.

The amphibian community of an agricultural wetland near Butare in southern Rwanda comprised 15 anuran species. Rarefaction and jackknife analyses corroborated that the complete current species richness of the assemblage had been recorded, and the results of acoustic niche analysis suggested species saturation of the community. Surveys at many other Rwandan localities showed that the species recorded in Butare are widespread in cultivated and pristine wetlands. The species were readily distinguishable using morphological, bioacoustic, and molecular (DNA barcoding) features, but only eight of the 15 species could be assigned unambiguously to nominal species. The remaining represented undescribed or currently unrecognized taxa, including three species of *Hyperolius*, two *Phrynobatrachus* species, one *Ptychadena* species, and one species of *Amietia*. The identities of the *Ptychadena* species and one of the *Hyperolius* species were addressed in the frame of this thesis (Chapters III and VI; see below). The identity of the Rwandan species of the *Hyperolius cinnamomeoventris* group is currently investigated in a larger study together with colleagues from the USA and the University of Trier (Bell *et al.*, in prep.), whereas the identities of the *Phrynobatrachus* species will be addressed in a comprehensive revision of the Rwandan species of the genus (Dehling & Sinsch, in prep.).

The diversity of the Ridged Frogs in Rwanda was investigated in two studies (Chapters III and IV). Three species of *Ptychadena* were recorded in wetlands in the catchment of the Nile. They can be distinguished by morphological characters (morphometrics and qualitative features) as well as by their advertisement calls and genetics. One of the species, *P. anchietae*, had not been previously recorded from Rwanda. The Rwandan species of the *P. mascareniensis* group was shown to differ from the topotypic population as well as from other genetic lineages in sub-Saharan Africa and an old available name, *P. nilotica*, was resurrected from synonymy for this lineage. The *P. mascareniensis* group will be further

investigated in a future study in collaboration with several colleagues. After the relationships within the group have been resolved, several of the lineages will probably be described as distinct species.

Two further *Ptychadena* species were identified among voucher specimens from Rwanda deposited in the collection of the RMCA, *P. chrysogaster* and *P. uzungwensis*. Morphologically they can be unambiguously distinguished from each other and the three other Rwandan species. A key based on qualitative morphological characters was developed, which allows unequivocal identification of specimens of all species that have been recorded from Rwanda. DNA was isolated from a Rwandan voucher specimen of *P. chrysogaster*, and the genetic analysis corroborated the species' distinct status. Attempts to record *P. chrysogaster* at sites, from which large voucher series are deposited in the RMCA, including the species' type locality (Lake Karago), remained unsuccessful. Vegetation at these sites has been converted quite recently from forest into farmland and it is possible that this has promoted population decline in *P. chrysogaster* and its replacement by *P. anchietae* and *P. nilotica*. These species had not been previously recorded at the sites but were commonly encountered by us. A recent invasion of agricultural sites by species of *Ptychadena* was also indicated by the results of the acoustic niche analysis (Chapter II). The current distribution of *P. chrysogaster* in Rwanda will be further investigated during future field work.

A species of *Hyperolius* collected in the Nyungwe National Park was compared to all other Rwandan species of the genus and to morphologically or genetically similar species from neighbouring countries (Chapter V). Its distinct taxonomic status was justified by morphological, bioacoustic, and molecular evidence and it was described as a new species, *H. jackie*.

The species of the *H. nasutus* group collected at agricultural sites in Rwanda (Chapter II) was described as a new species in the course of a revision of the species of the *Hyperolius nasutus* group (Chapter VI). The group was shown to consist of 15 distinct species which can be distinguished from each other genetically, bioacoustically, and morphologically.

The disc-fingered Reed Frog, *Hyperolius discodactylus*, was in the focus of two further studies of which the results are presented in Chapters VII and VIII. In Chapter VII, the species' aerial performance, i.e. parachuting, is described. It represents a novel observation of a behaviour that has been known from a number of Southeast Asian and Neotropical frog

species. Parachuting frogs, including *H. discodactylus*, exhibit certain morphological characteristics and, while airborne, assume a distinct posture which is best-suited for maneuvering in the air.

Chapter VIII presents the results of a study that addressed the validity of the taxon *H. alticola* which had been considered either a synonym of *H. discodactylus* or a distinct species. We examined the type material of both taxa and reassessed the status of *H. alticola* using morphological data from historic and new collections, call recordings, and molecular data from animals collected on recent expeditions. A northern and a southern genetic clade were identified, a divide that is weakly supported by diverging morphology of the vouchers from the respective localities. No distinction in advertisement call features could be recovered to support this split and both genetic and morphological differences between the two geographic clades are marginal and not always congruent and more likely reflect population-level variation. We therefore concluded that *H. alticola* is not a valid taxon and should be treated as a synonym of *H. discodactylus*.

During the field work for this thesis, five *Hyperolius* species were collected that have not yet been reported from Rwanda (*H. jackie*, *H. rwandae*, *H.* cf. *cinnamomeoventris*, *H. frontalis*, and *H. parallelus*). This brings the number of species recorded from the country from six to eleven. Some of these species have very similar advertisement calls. The systematic relationships and the call evolution within this group will be investigated in an upcoming paper (Dehling & Sinsch, in prep.) which will also include species from outside the Albertine Rift.

References

Adeba P. J., Kouassi P. & Rödel M.-O. (2010) Anuran amphibians in a rapidly changing environment - revisiting Lamto, Côte d'Ivoire, 40 years after the first herpetofaunal investigations. – African Journal of Herpetology 59: 1–16.

Ahl E. (1924) Neue afrikanische Frösche. – Zoologischer Anzeiger 61: 99–103.

Ahl E. (1925, "1923") Über neue afrikanische Frösche der Familie Ranidae. – Sitzungsberichte der Gesellschaft naturforschender Freunde zu Berlin 1923: 96–106.

Ahl E. (1929) Zur Kenntnis der afrikanischen Baumfrosch-Gattung *Leptopelis*. – Sitzungsberichte der Gesellschaft naturforschender Freunde zu Berlin 1929: 185–222.

Ahl E. (1930) Ueber die afrikanischen Arten der Baumfroschgattung *Megalixalus*. Sitzungsberichte der Gesellschaft naturforschender Freunde zu Berlin 1930: 89–102.

Ahl E. (1931a) Amphibia. Anura III. Polypedatidae. In: Schulze F. E. & Kükenthal W. (Eds.) Das Tierreich, 55. Lieferung. Walter de Gruyter: Berlin & Leipzig, pp. iii–xvi + 1–475.

Ahl E. (1931b) Zur Systematik der afrikanischen Arten der Baumfroschgattung *Hyperolius*. – Mitteilungen aus dem Zoologischen Museum in Berlin 17: 1–132.

Akef M. S. A. & Schneider H. (1995) Calling behavior and mating call pattern in the mascarene frog, *Ptychadena mascareniensis*, (Amphibia, Anura, Ranidae) in Egypt. – Journal of African Zoology 109: 225–229.

Amiet J.-L. (2005) Les *Hyperolius* camerounais du groupe d' *H. nasutus* (Amphibia, Anura, Hyperoliidae). – Revue Suisse de Zoologie 112: 271–310.

Amiet J.-L. (2006a) *Hyperolius igbettensis*. In: IUCN (2008) IUCN Red List of Threatened Species. Version 2008. <www.iucnredlist.org>. Downloaded on 9 January 2012.

Amiet J.-L. (2006b) *Hyperolius adspersus*. In IUCN (2008) IUCN Red List of Threatened Species. Version 2008. <www.iucnredlist.org>. Downloaded on 9 January 2012.

AmphibiaWeb (2014) Information on amphibian biology and conservation [web application]. Berkeley, California: AmphibiaWeb. Available at: http://amphibiaweb.org/. (accessed: April 18, 2014).

Andreone F., Channing A., Drewes R., Gerlach J., Glaw F., Howell K., Largen M., Loader S., Lötters S., Minter L., Pickersgill M., Raxworthy C., Rödel M.-O., Schiøtz A., Vallan D. & Vences M. (2008) Amphibians of the afrotropical realm. pp. 53-58 in: Stuart S. N., Hoffman M., Chanson J. S., Cox N. A., Berridge R. J., Ramani P. & Young B. E. (eds.) Threatened amphibians of the world. – Lynx Editions: Barcelona, Spain; IUCN: Gland Switzerland; and Conservation International: Arlington, Virginia. xv + 758 pp.

Anthony N. M., Johnson-Bawe M., Jeffery K., Clifford S. L., Abernethy K. A., Tutin C. E., Lahm S. A., White L. J. T., Utley J. F., Wickings E. J. & Bruford M. W. (2007) The role of Pleistocene refugia and rivers in shaping gorilla genetic diversity in central Africa. – Proceedings of the National Academy of Sciences of the United States of America 104: 20432–20436.

Arnoult J. & Lamotte M. (1958) Contribution à l'étude des batraciens de l'Ouest africain VI. Développement larvaire de deux espèces d'*Hyperolius*: *H. zonatus* LAURENT et *H. lamottei* LAURENT. – Bulletin de l'Institut Fondamental d'Afrique Noire, Serie A Sciences Naturelles 20: 573–586.

Baha el Din S. (2005) On the occurrence of *Ptychadena schillukorum* (Werner, 1907) in Egypt. – Herpetozoa 18: 178–181.

Baillie J. E. M., Hilton-Taylor C. & Stuart S. N. (eds.) (2004) 2004 IUCN Red List of Threatened Species. A Global Species Assessment. – IUCN: Gland, Switzerland and Cambridge, UK.

Barbour T. & Loveridge A. (1929) A new frog of the genus *Arthroleptis* from the Belgian Congo. – Proceedings of the New England Zoological Club 11: 25–26.

Barbour T. & Loveridge A. (1946) First supplement to typical reptiles and amphibians. – Bulletin of the Museum of Comparative Zoology at Harvard College 96 (2): 59–214.

Behangana M. & Luiselli L. (2008) Habitat niche community-level analysis of an amphibian assemblage at Lake Nabugabo, Uganda. – Web Ecology 8: 125–134.

Bell R. C. & Zamudio K. R. (2012) Sexual dichromatism in frogs: natural selection, sexual selection and unexpected diversity. – Proceedings of the Royal Society B: Biological Sciences 279: 4687–4693.

Benson D. A., Karsch-Mizrachi I., Lipman D. J., Ostell J. & Wheeler D. L. (2004) GenBank: update. – Nucleic Acids Research 32 (database issue): D23–D26.

Benson D. A., Karsch-Mizrachi I., Clark K., Lipman D. J., Ostell J. & Sayers E. W. (2012) GenBank. – Nucleic Acids Research 40 (database issue): D48–D53.

Benson D. A., Cavanaugh M., Clark K., Karsch-Mizrachi I., Lipman D. J., Ostell J. & Sayers E. W. (2013) GenBank. – Nucleic Acids Research 41 (database issue): 36–42.

Biju S. D. & Bossuyt F. (2003) New frog family from India reveals an ancient biogeographical link with the Seychelles. – Nature 425: 711–714.

Biju S. D., van Bocxlaer I., Mahoney S., Dinesh K. P., Radhakrishnan C., Zachariah A., Giri V. & Bossuyt F. (2011) A taxonomic review of the Night Frog genus *Nyctibatrachus* Boulenger, 1882 in the Western Ghats, India (Anura: Nyctibatrachidae) with description of twelve new species. – Zootaxa 3029: 1–96.

Blackburn D. C. (2008) Biogeography and evolution of body size and life history of African frogs: Phylogeny of squeakers (*Arthroleptis*) and long-fingered frogs (*Cardioglossa*) estimated from mitochondrial data. – Molecular Phylogenetics and Evolution 49: 806–826.

Blackburn D. C. (2009) Diversity and evolution of male secondary sexual characters in African squeakers and long-fingered frogs. – Biological Journal of the Linnean Society 96: 553–573.

Bocage J. V. B. d. (1886) Reptiles et batraciens nouveaux de l'Ile de St. Thomé. – Jornal de sciências, mathemáticas, physicas e naturaes 11: 71–75.

Böckle M., Preininger D. & Hödl W. (2009) Communication in noisy environments I: Acoustic signals of *Staurois latopalmatus* Boulenger 1887. – Herpetologica 65: 154–165.

Boquimpani-Freitas L., Marra R. V., van Sluys M. & Duarte Rocha C. F. (2007) Temporal niche of

acoustic activity in anurans: interspecific and seasonal variation in a neotropical assemblage from south-eastern Brazil. – Amphibia-Reptilia 28: 269–276.

Bossuyt F. & Milinkovitch M. C. (2000) Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. – Proceedings of the National Academy of Sciences of the United States of America 97: 6585–6590.

Boulenger G. A. (1879) Synonymie de *Rana mascareniensis*. – Bulletin de la Société Zoologique de France 4: 92–94.

Boulenger G. A. (1917) Descriptions of new frogs of the genus *Rana*. – The Annals and Magazine of Natural History, including Zoology, Botany, and Geology, Series 8, 20: 413–418.

Branch B. (2005) A Photographic Guide to Snakes, other reptiles and amphibians of East Africa. – Struik Publishers: Cape Town.

Burgess N., Hales J. D., Underwood E., Dinerstein E., Olson D., Itoua I., Schipper J., Ricketts T. & Newman K. (2004) Terrestrial ecoregions of Africa and Madagascar. – Island Press: Washington, DC, USA.

Bwong B. A., Chira R., Schick S., Veith M. & Lötters S. (2009) Diversity of Ridged Frogs (Ptychadenidae: *Ptychadena*) in the easternmost remnant of the Guineo-Congolian rain forest: an analysis using morphology, bioacoustics and molecular genetics. – Salamandra 45: 129–146.

Channing A. (2001) Amphibians of central and southern Africa. – Comstock Publishing Associates: Ithaca and London.

Channing A. & Howell K. M. (2006) Amphibians of East Africa. – Cornell University Press: Ithaca, USA, and London, UK; and Edition Chimaira: Frankfurt, Germany.

Channing A., Moyer D. & Burger M. (2002) Cryptic species of sharp-nosed reed frogs in the *Hyperolius nasutus* complex: advertisement call differences. – African Zoology 37: 91–99.

Channing A., Hillers A., Lötters S., Rödel M.-O., Schick S., Conradie W., Rödder D., Mercurio V., Wagner P., Dehling J. M., du Preez L. H., Kielgast J. & Burger M. (2013) Taxonomy of the super-cryptic *Hyperolius nasutus* group of long reed frogs of Africa (Anura: Hyperoliidae), with descriptions of six new species. – Zootaxa 3620: 301–350.

Clement M., Posada D. & Crandall K. A. (2000) TCS: a computer program to estimate gene genealogies. – Molecular Ecology 9: 1657–1660.

Collar N. J. & Stuart S. N. (1988) Key forests for threatened birds in Africa. ICBP Monograph 3: 1–102.

Colwell R. K. (2009) EstimateS: Statistical estimation of species richness and shared species from samples. Version 8.2. – User's Guide and application published at: http://purl.oclc.org/estimates.

Colwell R. K. & Futuyama D. J. (1971) On the measurement of niche breadth and overlap. – Ecology 52: 567–576.

Conradie W., Branch W. R. & Tolley K. A. (2013) Fifty shades of grey: giving colour to the poorly known Angolan Ashy reed frog (Hyperoliidae: *Hyperolius cinereus*), with the description of a new species. – Zootaxa 2636: 201–223.

Conradie W., Branch W. R., Measey G. J. & Tolley K. A. (2012) A new species of *Hyperolius* Rapp, 1842

(Anura: Hyperoliidae) from the Serra da Chela mountains, south-western Angola. – Zootaxa 3269: 1–17.

Crump M. L. (1974) Reproductive strategies in a tropical anuran community. – Miscellaneous Publication of the Museum of Natural History, The University of Kansas 61: 1–68.

de Witte G.-F. (1933) Reptiles récoltés au Congo Belge par le Dr. H. Schouteden et par M. G.-F. de Witte. – Annales du Musée du Congo Belge, Zoologie (C) Série I 3(2): 52–100.

de Witte G.-F. (1934) Batraciens récoltés au Congo Belge par le Dr. H. Schouteden et par M. G.-F. de Witte. – Annales du Musée du Congo Belge, Zoologie (C) Série I 3(4): 153–188.

de Witte G.-F. (1941) Batraciencs et Reptiles. – Exploration du Parc National Albert, Mission G. F. de Witte (1933-1935) 33: 1–261, 76 plts.

de Witte G.-F. (1953) Reptiles. – Exploration du Parc National de l'Upemba, Mission G. F. de Witte (1946-1949) 8: 5–322, xli plates.

de Witte G.-F. (1962) Genera des serpents du Congo et du Ruanda-Urundi. – Annales Sciences Zoologiques, Musée Royal de l'Afrique Centrale 104: i–viii + 1–203, xv plates.

de Witte G.-F. (1965) Les caméléons de l'Afrique centrale: République démocratique du Congo, République du Rwanda et Royaume du Burundi. – Annales du Musée de l'Afrique Centrale - Serie 8° - Sciences Zoologiques 142, 217 pp.

de Witte G.-F. & Laurent R. (1942) Contribution à la faune herpétologique du Congo belge. – Revue de Zoologie et Botanique africaines 36: 101–115.

Dehling J. M. (2012a) An African glass frog: a new *Hyperolius* species (Anura: Hyperoliidae) from Nyungwe National Park, southern Rwanda. – Zootaxa 3391: 52–64.

Dehling J. M. (2012b) *Hyperolius discodactylus* (Disc-fingered Reed Frog). Parachuting. – Herpetological Review 43: 463.

Dehling J. M. & Matsui M. (2013) A new species of *Leptolalax* (Anura: Megophryidae) from Gunung Mulu National Park, Sarawak, East Malaysia (Borneo). – Zootaxa 3670: 33–44.

Dehling J. M. & Sinsch U. (2013) Diversity of Ridged Frogs (*Ptychadena* spp.) in wetlands of the upper Nile in Rwanda: Morphological, bioacoustic, and molecular evidence. – Zoologischer Anzeiger 253: 143–157. http://dx.doi.org/10.1016/j.jcz.2013.08.005.

di Tada I. E., Martino A. & Sinsch U. (2001) Release vocalizations in neotropical toads (*Bufo*): ecological constraints and phylogenetic implications. – Journal of Zoological Systematics and Evolutionary Research 39: 13–23.

Diamond A. W. & Hamilton A. C. (1980) The distribution of forest passerine birds and Quaternary climatic change in tropical Africa. – Journal of Zoology 191: 379–402.

Drewes R. C. (1984) A phylogenetic analysis of the Hyperoliidae (Anura): treefrogs of Africa, Madagascar and the Seychelles Islands. – Proceedings of the California Academy of Sciences 139: 1–70.

Drewes R. C. & Vindum J. V. (1994) Amphibians of the Impenetrable Forest, southwest Uganda. – Journal of African Zoology 108: 55–70.

Drewes R. C. & Wilkinson J. A. (2004) The California Academy of Sciences Gulf of Guinea expedition

(2001) I. The taxonomic status of the genus *Nesionixalus* Perret, 1976 (Anura: Hyperoliidae), treefrogs of São Tomé and Príncipe, with comments on the genus *Hyperolius*. – Proceedings of the California Academy of Sciences 55: 1–13.

Dudley R., Byrnes G., Yanoviak S. P., Borrell B., Brown R. M. & McGuire J. A. (2007) Gliding and the functional origins of flight: biomechanical novelty or necessity? – Annual Review of Ecology, Evolution, and Systematics 38: 179–201.

Duellman W. E. (1970) The hylid frogs of Middle America. Volume 1. – Monograph of the Museum of Natural History, University of Kansas 1: 1–753.

Duellman W. E. (1978) The biology of an equatorial herpetofauna in Amazonian Ecuador. – Miscellaneous Publication of the Museum of Natural History, The University of Kansas 65: 1–352.

Duméril A. M. C. & Bibron G. (1841) Erpétologie générale ou histoire naturelle complète des reptiles. Volume 8. Librarie Enclyclopedique de Roret: Paris.

du Preez L. & Carruthers V.(2009) A complete guide to the frogs of southern Africa. – Struik Nature: Cape Town.

Edgar R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. – Nucleic Acids Research 32 (5): 1792–1797.

Emerson S. B. & Koehl M. A. R. (1990) The interaction of behavioral and morphological change in the evolution of a novel locomotor type: „flying" frog. – Evolution 44: 1931–1946.

Evans B. J., Kelley D. B., Tinsely R. C., Melnick D. J. & Cannatella D. C. (2004) A mitochondrial DNA phylogeny of African clawed frogs: phylogeography and implications for polyploid evolution. – Molecular Phylogenetics and Evolution 33: 197–213.

Evans B. J., Carter T. F., Tobias M. L., Kelley D. B., Hanner R. & Tinsley R. C. (2008) A new species of clawed frog (genus *Xenopus*) from the Itombwe Massif, Democratic Republic of the Congo: implications for DNA barcodes and biodiversity conservation. – Zootaxa 1780: 55–68.

Evans B. J., Greenbaum E., Kusamba C., Carter T. F., Tobias M. L., Mendel S. A. & Kelley D. B. (2011) Description of a new octoploid frog species (Anura: Pipidae: *Xenopus*) from the Democratic Republic of the Congo, with a discussion of the biogeography of African clawed frogs in the Albertine Rift. – Journal of Zoology 283: 276-290.

Fischer E. & Hinkel H. (1992) Natur Ruandas / La Nature du Rwanda. Einführung in die Flora und Fauna Ruandas und ihre wichtigsten Biotopsysteme. – Ministerium des Inneren und für Sport, Rheinland-Pfalz: Mainz.

Fischer E. & Killmann D. (2008) Illustrated field guide to the plants of Nyungwe National Park Rwanda. – Koblenz Geographical Colloquia, Series Biogeographical Monographs 1: 1–772.

Fjeldså J. & Lovett J. C. (1997) Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. – Biodiversity and Conservation 6: 325–346.

Fouquet A., Gilles A., Vences M., Marty C., Blanc M. & Gemmell N. J. (2007) Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. – PLoS ONE 2: e1109.

Frétey T., Dewyner M. & Blanc C. P. (2011) Amphibiens d'Afrique central et d'Angola. Clé de determination illustrée des amphibiens du Gabon et du Mbini. – Biotope, Meze (Collection Parthénope), Muséum national d'Histoire naturelle: Paris.

Frost D. R. (1985) Amphibian species of the world: a taxonomic and geographical reference. – Association of Systematics Collection and Allen Press, Kansas, USA, 732 pp.

Frost D. R. (2011) Amphibian Species of the World: an Online Reference. Version 5.5 (31 December 2011). – American Museum of Natural History, New York, USA, accessible at http://research.amnh.org/vz/herpetology/amphibia/.

Frost D. R. (2013) Amphibian Species of the World: an Online Reference. Version 5.6. – American Museum of Natural History, NY, USA. Available from http://research.amnh.org/herpetology/amphibia/index.html (last accessed 26 August 2013).

Frost D. R., Grant T., Faivovich J., Bain R. H., Haas A., Haddad C. F. B., de Sa R. O., Channing A., Wilkinson M. & Donnellan S. C. (2006) The amphibian tree of life. – Bulletin of the American Museum of Natural History 297: 1–291.

Garcia-Rutledge E. J. & Narins P. M. 2001. Shared acoustic resources in an old world frog community. – Herpetologica 57: 104–116.

Goebel A. M., Donnelly J. M. & Atz M. E. (1999) PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome b in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians sucessfully. – Molecular Phylogenetics & Evolution 11: 163–199.

Gottsberger B. & Gruber E. (2004) Temporal partitioning of reproductive activity in a neotropical anuran community. – Journal of Tropical Ecology 20: 271–280.

Gower D. J., Papadopoulou A., Doherty-Bone T. M., Pupin F., San Mauro D., Loader S. & Wilkinson M. (2011) The systematics of *Boulengerula fischeri* (Amphibia: Gymnophiona: Caeciliidae) based on morphological and molecular data. – Zootaxa 2767: 14–24.

Greenbaum E., Sinsch U., Lehr E., Valdez F. & Kusamba C. (2013) Phylogeography of the reed frog *Hyperolius castaneus* (Anura: Hyperoliidae) from the Albertine Rift of Central Africa: Implications for taxonomy, biogeography and conservation. – Zootaxa 3731: 473–494.

Guibé J. & Lamotte M. (1957) Révision systématique des *Ptychadena* (Batraciens, Anoures, Ranidés) d'Afrique occidentale. – Bulletin de l'Institut Française d'Afrique Noire. Série A, Sciences Naturelles 19: 937–1003.

Hijmans R. J., Cameron S. E., Parra J. L., Jones P. G. & Jarvis A. (2005) Very high resolution interpolated climate surfaces for global land areas. – International Journal of Climatology 25: 1965–1978.

Hijmans R. J., Phillips S.,Leathwick J. & Elith J. (2012) Dismo: Species distribution modelling. – R package version 0.7-17. http://CRAN.R- project.org/package=dismo.

Hillis D. M. & Bull J. J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. – Systematic Biology 42: 182–192.

Hillis D. M., Mable B. K., Larson A., Davis S. K. & Zimmer E. A. (1996) Nucleic acids IV: Sequencing and cloning. In: Hillis D. M., Moritz C. &. Mable B. K (eds.) Molecular Systematics. 2nd ed. – Sinauer

Associates: Sunderland, Massachusetts, USA.

Hinkel H. (1993) Zur Biogeographie und Ökoethologie der Reptilienfauna von montanen Feuchtwäldern in Ruanda und Ost-Zaire unter Berücksichtigung der Amphibien. – Dissertation, Universität Mainz, 10 (unnumbered) + 205 pp.

Hinkel H. (1996) Zur Flora und Fauna der Wälder Ruandas/Zentralafrika. III. Der Nyungwe-Wald. Teil 2: Kommentierte Artenliste der Herpetofauna des Nyungwe-Waldes mit Neunachweisen. – Mainzer naturwissenschaftliches Archiv 34: 55–81.

Holt R. D. (2009) Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. – Proceedings of the National Academy of Sciences of the United States of America 106: 19659–19665.

Hölting M., Böhme W. & Schmitz A. (2009) Range extension for the tree frog *Leptopelis karissimbensis* Ahl, 1929 in Rwanda (Anura: Arthroleptidae). – Herpetology Notes 2:45–47.

Hutchison G. E. (1957) Concluding remarks. – Cold Spring Harbor Symposia on Quantitative Biology 22: 415–427.

IUCN (2011) The IUCN Red List of Threatened Species. Version 2011.2 <www.iucnredlist.org>. Downloaded on 06 April 2012.

IUCN (2013) The IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 21 November 2013.

Katariya V. & Chanson J. (2008) Amphibians and humans sharing only one planet. pp. 45-46 in: Stuart S. N., Hoffman M., Chanson J. S., Cox N. A., Berridge R. J., Ramani P. & Young B.E. (eds.) Threatened amphibians of the world. – Lynx Editions: Barcelona, Spain; IUCN: Gland Switzerland; and Conservation International: Arlington, Virginia. xv + 758 pp.

Kocher T. D., Thomas W. K., Meyer A., Edwards S. V., Pääbo S., Villablanca F. X. & Wilson A. C. (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. – Proceedings of the National Academy of Sciences of the United States of America 86: 6196–6200.

Köhler J., Scheelke K., Schick S., Veith M. & Lötters S. (2005a) Contribution to the taxonomy of hyperoliid frogs (Amphibia: Anura: Hyperoliidae): advertisement calls of twelve species from East and Central Africa. – African Zoology 40: 127–142.

Köhler J., Vietes D. R., Bonett R. M., Hita Garcia F., Glaw F., Steinke D. & Vences M. (2005b) New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate group. – BioScience 55: 693–696.

Lamotte M. (1967) Le problème des *Ptychadena* (Fam. Ranidae) du groupe *mascareniensis* dans l'ouest Africain. – Bulletin du Muséum National d'Histoire Naturelle, 2e series 39: 647–656.

Laurent R. F. (1940) Nouveaux batraciens congolais du genre *Hyperolius*. Revue de Zoologie et de Botanique Africaines 34: 1–7.

Laurent R. (1943) Les *Hyperolius* (Batraciens) du Musée du Congo. – Annales du Musée du Congo Belge, C.- Zoologie, Poissons, Reptiles, Amphibies Serie 1, 4: 61–140.

Laurent R. F. (1941, "1940") Contribution à l'ostéologie et à la systématique des ranides africains.

Première note. – Revue de Zoologie et de Botanique Africaines 34: 74–96.

Laurent R. F. (1947) Sur quelque Rhacophorides nouveaux du Parc National Albert appartenant aux genres *Megalixalus* et *Hyperolius*. – Bulletin de Musée Royal d'Histoire Naturelle de Belgique 23 (7): 1–8.

Laurent, R. (1950a) Diagnoses préliminaires de treize batraciens nouveaux d'Afrique centrale. – Revue de Zoologie et de Botanique Africaines 44: 1–18.

Laurent, R.F. (1950b) Genres *Afrixalus* et *Hyperolius* (Amphibia Salientia). Exploration du Parc National Albert. Mission G. F. de Witte (1933-1935). Institut des Parcs Nationaux du Congo Belge, Brussels, Belgium, 149 pp.

Laurent R. (1951) Deux reptiles et onze batraciens nouveaux d'Afrique centrale. – Revue de Zoologie et de Botanique Africaines 44: 360–381.

Laurent R. F. (1952) Reptiles et batraciens nouveaux de la région des Grands Lacs africains. – Revue de Zoologie et de Botanique Africaines 46: 269–279.

Laurent R. (1954) Etude de quelques espèces méconnues du genre *Ptychadena*. – Annales du Musée Royal du Congo Belge. Sciences Zoologiques 34: 1–34.

Laurent R. F. (1956a) Esquisse d'une faune herpéologique du Ruanda-Urundi. – Les Naturalistes Belges 1956: 280–287.

Laurent R. F. (1956b) Identite veritable de *Kassina senegalensis* et rehabilitation de *Kassina argyreivittis* Peters. – Folia Scientifica Africae Centralis 2: 17–18.

Laurent R. F. (1957) Notes sur les Hyperoliidae. – Revue de Zoologie et de Botanique Africaines 56: 274–282.

Laurent R. F. (1961) Note sur les *Hyperolius* et quelques *Afrixalus* (Salientia) du Musée de Berlin. – Revue de Zoologie et de Botanique Africaines 64: 65–96.

Laurent R. F. (1964) Museu do Dundo — Subsídios para o estudo da biologia na Lunda. Reptiles et Amphibiens de l'Angola (Troisème contribution). – Publicações Culturais da Companhia de Diamantes de Angola 67: 11–165.

Laurent R. F. (1972a) Amphibiens. – Exploration du Parc National des Virunga. Deuxième série, 22: 1–125, 11 plts.

Laurent R. F. (1972b) Caractères biométriques de trois genres monotypiques de la famille des Hyperoliidae. – Bulletin des Séances 1971–72: 256–275.

Laurent R. F. (1973) Le genre *Leptopelis* Günther (Salientia) au Zaïre. – Annales du Musée Royal de l'Afrique Centrale 202: 1–62.

Laurent R. F. (1976) Les genres *Cryptothylax*, *Phlyctimantis* et *Kassina* au Zaïre. – Annales du Musée Royal de l'Afrique Centrale. Série in Octavo, Science Zoologique 213: 1–67.

Laurent R. F. (1983a) About the herpetofauna of Central African montane forest. pp. 350–358 in: Rhodin A. G. J. & Miyata K. (eds.) Advances in herpetology and evolutionary biology: essays in honor of Ernest E. Williams. – Museum of Comparative Zoology: Cambridge, MA, USA.

Laurent R. F. (1983b) La superespèce *Hyperolius viridiflavus* (Duméril & Bibron, 1841) (Anura Hyperoliidae) en Afrique Centrale. – Monitore Zoologico Italiano. Nuova Serie, Supplemento

18: 1–93.

Linder H. P. 2001. Plant diversity and endemism in sub-Saharan tropical Africa. – Journal of Biogeography 28: 169–182.

Loader S. P., Ceccarelli F. S., Menegon M., Howell K. M., Kassahun R., Mengistu A. A., Saber S. A., Gebresenbet F., de Sá R., Davenport T. R. B., Larson J. G., Müller H., Wilkinson M. & Gower D. J. (2014, in press) Persistence and stability of Eastern Afromontane forests: evidence from brevicipitid frogs. – Journal of Biogeography, first published online: 28 April 2014, DOI: 10.1111/jbi.12331.

Loader S. P., Ceccarelli F. S., Wilkinson M., Menegon M., Mariaux J., de Sá R. O., Howell K. M. & Gower D. J. (2013) Species boundaries and biogeography of East African torrent frogs of the genus *Petropedetes* (Amphibia: Anura: Petropeditidae). – African Journal of Herpetology 62: 40–48.

Loader S. P., Poynton J. C., Lawson L., Blackburn D. C. & Menegon M. (2011) Herpetofauna of montane areas of Tanzania. 3. Amphibian diversity in the northwestern Arc Mountains, with the description of a new species of *Arthroleptis* (Anura: Arthroleptidae). – Fieldiana Life and Earth Sciences 4: 90–102.

Lode T. & le Jacques D. (2003) Influence of advertisement calls on reproductive success in the male midwife toad *Alytes obstetricans*. – Behaviour 140: 885–898.

Lötters A., Schick S., Scheelke K., Teege P., Kosuch J., Rotich D. & Veith M. (2004) Bio-sketches and partitioning of sympatric reed frogs, genus *Hyperolius* (Amphibia; Hyperoliidae), in two humid tropical African forest regions. – Journal of Natural History 38: 1969–1997.

Loveridge A. (1932) New reptiles and amphibians from Tanganyika Territory and Kenya Colony. – Bulletin of the Museum of Comparative Zoology 72: 375–387.

Loveridge A. (1933) Scientific results of an expedition to rain forest regions of eastern Africa. IX. Zoogeography and iternerary. – Bulletin of the Museum of Comparative Zoology 79: 481– 541.

Loveridge A. (1957) Check list of the reptiles and amphibians of East Africa (Uganda; Kenya; Tanganyika; Zanzibar). – Bulletin of the Museum of Comparative Zoology at Harvard College 117 (2): 1–362.

Martino A. L. & Sinsch U. (2002) Speciation by polyploidy in *Odontophrynus americanus*. – Journal of Zoology 257: 67–81.

Masters B. C., Fan V. & Ross H. A. (2011) Species delimitation: a geneious plugin for the exploration of species boundaries. – Molecular Ecology Resources 11 (1): 154–157.

May R. M. & MacArthur R. H. (1972) Niche overlap as a function of environmental variability. – Proceedings of the National Academy of Sciences of the United States of America 69: 1109–1113.

Mayr E. & O'Hara R. J. (1986) The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. – Evolution 40: 55–67.

Measey G. J., Hinkel H. H., Dumbo B. & Fischer E. (2011) Rediscovery of *Boulengerula fischeri*, with notes on its morphology and habitat. – African Journal of Herpetology 60: 47–59.

Measey G. J., Vences M., Drewes R. C., Chiari Y., Melo M. & Bourles B. (2007) Freshwater paths across the ocean: molecular phylogeny of the frog *Ptychadena newtoni* gives insights into amphibian colonization of oceanic islands. – Journal of Biogeography 34: 7–20.

Mendelson J. R. III, Savage J. M., Griffith E., Ross H., Kubicki B. & Gagliardo R. (2008) Spectacular new gliding species of *Ecnomiohyla* (Anura: Hylidae) from Central Panama. – Journal of Herpetology 42: 750–759.

Menegon M. (2008) Amphibians and reptiles of Nyungwe National Park: A preliminary report. – Unpublished report for the Wildlife Conservation Society, 4pp.

Menegon M. & Salvidio S. (2005) Amphibian and reptile diversity in the southern Udzungwa scarp forest reserve, south-eastern Tanzania. pp. 205-212 in: Huber B. A., Sinclair B. J. & Lampe K. H. (eds.) African Biodiversity: Molecules, Organisms, Ecosystems. – Springer Verlag: Berlin.

Mercurio V. (2011) Amphibians of Malawi. – Edition Chimaira: Frankfurt am Main.

Moritz C. & Cicero C. (2004) DNA barcoding: promise and pitfalls. – PLoS Biology 2(10): e354.

Murdoch D. J. & Chow E. D. (1996) A graphical display of large correlation matrices. – The American Statistician 50 (2): 178–180.

Myers C. W. & Duellman W. E. (1982) A new species of *Hyla* from Cerro Colorado, and other treefrog records and geographical notes from western Panama. – American Museum Novitates 2752: 1–32.

Napoleon Le Grand (1809) Description de l'Égypte, ou recueil des observations et des recherches qui ont été faites en Égypte pendant l'expédition de l'armée francaise. Histoire Naturelle, Tome premier. – L'Imprimerie imperiale: Paris.

Nieden F. (1911, "1910") Neue ostafrikanische Frösche aus dem Kgl. Zool. Museum in Berlin. Sitzungsberichte der Gesellschaft naturforschender Freunde zu Berlin 1910: 436–441.

Nieden F. (1913) Amphibia. pp. 165–195, 1 plt. in: Schubotz H. (Ed.) Wissenschaftliche Ergebnisse der Deutschen Zentral-Afrika-Expedition 1907–1908 unter Führung Adolf Friedrichs, Herzogs zu Mecklenburg. Band IV, Zoologie II. – Klinkhardt & Biermann: Leipzig.

Nix H. (1986) A biogeographic analysis of Australian elapid snakes. pp. 4–15 in: Longmore R (ed.) Atlas of elapid snakes of Australia. – Bureau of Flora and Fauna: Canberra.

Noonan B. P. & Gaucher P. (2005) Phylogeography and demography of Guianan harlequin toads (*Atelopus*): diversification within a refuge. – Molecular Ecology 14: 3017–3031.

Nussbaum R. A. & Hinkel H. (1994) Revision of East African caecilians of the genera *Afrocaecilia* Taylor and *Boulengerula* Tornier (Amphibia: Gymnophiona: Caeciliaidae). – Copeia 1994: 750–760.

Odierna G., Aprea G., Andreone F., Böhme W. & Vences M. (2007) Cytosystematics of hyperoliid frogs: Phylogeny of *Heterixalus*, low karyotypic variability in hyperoliines and separate phylogenetic position of *Leptopelis*. – Italian Journal of Zoology 74 (1): 71–81.

Oksanen J., Blanchet F. G., Kindt R., Legendre P., Minchin P. R., O'Hara R. B., Simpson G. L., Oksanen M. J. & Suggests M. (2013) Vegan: community ecology package. – R package version 2.0-8.

Oliver J. A. (1951) „Gliding" in amphibians and reptiles, with a remark on an arboreal adaptation in

the lizard, *Anolis carolinensis carolinensis* Voigt. – The American Naturalist 85: 171–176.

Padial J. M., Miralles A., de la Riva I. & Vences M. (2010) The integrative future of taxonomy. – Frontiers in Zoology 7: article 16.

Paepke H. J. (2013) Ernst Ahl (1898 1945), sein Leben und seine Bedeutung für die Herpetologie und Terrarienkunde. – Sekretär 13 (1): 15–46.

Palumbi S. R. (1996) Nucleic acids II: the polymerase chain reaction. pp. 205–247 in: Hillis D. M., Moritz C. & Mable B. K. (eds.) Molecular Systematics. – Sinauer Associates, Inc.: Sunderland.

Palumbi S., Martin A., Romano S., McMillan W. O., Stice L. & Grabowski G. (2002) The simple fool's guide to PCR, Version 2. – University of Hawaii: Honolulu, Hawaii, 46 pp.

Passmore N. I. (1977) Mating calls and other vocalizations of five species of *Ptychadena* (Anura: Ranidae). – South African Journal of Science 173: 212–214.

Perret J.-L. (1976) Revision des amphibiens Africaines et principalement des types conservés au Musée Bocage de Lisbonne. – Arqivos do Museu Bocage ser 2, 6: 15–34.

Perret J.-L. (1979) Remarques et mise au point sur quelques espèces de *Ptychadena* (Amphibia, Ranidae). – Bulletin de la Société neuchâteloise des Sciences naturelles 102: 5–21.

Peters W. (1863) Bemerkungen über verschiedene Batrachier, namentlich uber [sic] die Originalexemplare der von Schneider und Wiegmann beschriebenen Arten des zoologischen Museums zu Berlin. – Monatsberichte der Königlich Preussischen Akademie der Wissenschaften zu Berlin. Aus dem Jahre 1863: 76–82.

Pianka E. R. (1986) Ecology and natural history of desert lizards. – Princeton Univ. Press: New Jersey.

Pickersgill M. (2007) Frog search - results of expeditions to southern and eastern Africa. – Chimaira: Frankfurt am Main.

Plumptre A. J., Davenport T. R. B., Behanyana M., Kityo R., Eilu G., Ssegawa P., Ewango C., Meirte D., Kahindo C., Herremans M., Kerbis Peterhans J., Pilgrim J. D., Wilson M., Languy M. & Moyer D. (2007) The biodiversity of the Albertine Rift. – Biological Conservation 134: 178–194.

Poynton J. C. (1964) The amphibians of southern Africa: a faunal study. – Annals of the Natal Museum 17: 1–334.

Poynton J. C. (1970) Guide to the *Ptychadena* (Amphibia: Ranidae) of the southern third of Africa. – Annals of the Natal Museum 20: 365–375.

Poynton J. C. & Boycott R. C. (1996) Species turnover between afromontane and eastern African lowland faunas: Patterns shown by amphibians. – Journal of Biogeography 23: 669– 680.

Poynton J. C. & Broadley D. G. (1985) Amphibia Zambesiaca 2. Ranidae. – Annals of the Natal Museum 27: 115–181.

Poynton J. C. & Broadley D. G. (1987) Amphibia Zambesiaca 3. Rhacophoridae and Hyperoliidae. – Annals of the Natal Museum 28: 161–229.

Poynton J. & Channing A. (2004) *Ptychadena grandisonae*. In: IUCN (2012) IUCN Red List of Threatened Species. Version 2012.2. <www.iucnredlist.org>.

Poynton J., Largen M., Howell K., Channing A., Minter L. & Lötters S. (2004) *Ptychadena anchietae*. In:

IUCN (2012) IUCN Red List of Threatened Species. Version 2012.2. <www.iucnredlist.org>.

Poynton J. C., Loader S. P., Sherratt E. & Clarke B. T. (2007) Amphibian diversity in east african biodiversity hotspots: Altitudinal and latitudinal pattern. – Biodiversity and Conservation 16: 1103–1118.

Prado C. P. D., Uetanabaro M. & Haddad C. F. B. (2005) Breeding activity patterns, reproductive modes, and habitat use by anurans (Amphibia) in a seasonal environment in the Pantanal, Brazil. –Amphibia-Reptilia 26: 211–221.

Preininger D., Böckle M. & Hödl W. (2007) Comparison of anuran acoustic communities of two habitat types in the Danum Valley Conservation Area, Sabah, Malaysia. – Salamandra 43: 129–138.

Pupin F., Hügli D., Loader S., Sekisambu R. & Menegon M. (2013) Baseline for amphibian monitoring in some Albertine Rift sites. Comprehensive monitoring for climate change adaptation and management in the Albertine Rift Protected Area Network. – Unpublished report, 66 pp.

R Core Team (2013) R: A language environment for statistical computing. – R Foundation for statistical computing, Vienna, Austria. http://www.R-project.org

Rambaut A. & Drummond A. J. (2007) Tracer version 1.4. Computer program and documentation distributed by the author. http://beastbioedacukTracer (accessed 26 August 2013).

REMA (2009) Rwanda State of Environment and Outlook Report. – Rwanda Environment Management Authority (REMA), Kigali, Rwanda.

Richards C. M. & Moore W. S. (1996) A phylogeny for the African treefrog family Hyperoliidae based on mitochondrial rDNA. – Molecular Phylogenetics and Evolution 5 (3): 522–532.

Richards C. M. & Schiøtz A. (1977) A new species of reed frog, *Hyperolius cystocandicans*, from montane Kenya. – Copeia 1977: 285–294.

Ricklefs R. E. (2010) Evolutionary diversification, coevolution between populations and their antagonists, and the filling of niche space. – Proceedings of the National Academy of Sciences of the United States of America 107: 1265–1272.

Roberts E. M., Stevens N. J., O'Connor P. M., Dirks P. H. G. M., Gottfried M. D., Clyde W. C., Armstrong R. A., Kemp A. I. S. & Hemming S. (2012) Initiation of the western branch of the East African Rift coeval with the eastern branch. – Nature Geoscience 5: 289–294.

Rödel M.-O. (2000) Herpetofauna of West Africa, Vol. I: Amphibians of the West African savanna. – Edition Chimaira: Frankfurt am Main.

Rödel M.-O. & Agyei A. C. (2003) Amphibians of the Togo-Volta highlands, eastern Ghana. – Salamandra 39: 207–234.

Rödel M.-O. & Ernst R. (2003) The amphibians of Marahoué and Mont Péko National Parks, Ivory Coast. – Herpetozoa 16: 23–39.

Rödel M.-O. & Schiøtz A. (2004) *Hyperolius lamottei*. In: IUCN (2009) IUCN Red List of Threatened Species. Version 2009.1. <www.iucnredlist.org>. Downloaded on 19 January 2012.

Rödel M.-O., Bangoura M. A. & Böhme W. (2004) The amphibians of south-eastern Republic of Guinea (Amphibia: Gymnophiona, Anura). – Herpetozoa 17: 99–118.

Rödel M.-O., Kosuch J., Grafe T. U., Boistel R., Assemian N. E., Kouamé N. G., Tohé B., Gourène G., Perret J.-L., Henle K., Tafforeau P., Pollet N. & Veith M. (2009) A new tree-frog genus and species from Ivory Coast, West Africa (Amphibia: Anura: Hyperoliidae). – Zootaxa 2044: 23–45.

Rödel M.-O., Lampert K. P. & Linsenmair K. E. (2006) Reproductive biology of the West African savannah frog *Hyperolius nasutus* Günther, 1864 (Amphibia: Anura: Hyperoliidae). – Herpetozoa 19: 3–12.

Rödel M.-O., Sandberger L., Penner J., Mané Y. & Hillers A. (2010) The taxonomic status of *Hyperolius spatzi* Ahl, 1931 and *Hyperolius nitidulus* Peters, 1875 (Amphibia: Anura: Hyperoliidae). – Bonn zoological Bulletin 57: 177–188.

Roelke C. E. & Smith E. N. (2010) Herpetofauna, Parc National des Volcans, North Province, Republic of Rwanda. – Check List 6: 525–531.

Roelke C. E., Mehdibeigi R. & Smith E. N. (2009) Tadpole of the frog, *Leptopelis karissimbensis*, from Rwanda (Anura: Arthroleptidae). – Journal of Herpetology 43: 362–366.

Roelke C. E., Greenbaum E., Kusamba C., Aristote M. M. & Smith E. N. (2011) Systematics and conservation status of two distinct Albertine Rift treefrogs, *Leptopelis karissimbensis* and *Leptopelis kivuensis* (Anura: Arthroleptidae). – Journal of Herpetology 45: 343–351.

Ronquist F., Teslenko M., van der Mark P., Ayres D. L., Darling A., Hohna S., Larget B., Liu L., Suchard M. A. & Huelsenbeck J. P. (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. – Systematic Biology 61 (3): 539–542.

ROR (2008) Strategic Plan for the Transformation of Agriculture in Rwanda - Phase II (PSTA II). Final Report. – Ministry of Agriculture and Animal Resources, Republic of Rwanda (ROR): Kigali.

Roy M. S., Sponer R. & Fjeldså J. (2001) Molecular systematics and evolutionary history of Akalats (genus *Sheppardia*): A pre-Pleistocene radiation in a group of African forest birds. – Molecular Phylogenetics and Evolution 18: 74–83.

Savage J. & Heyer W. (1997) Digital webbing formulae for anurans: a refinement. – Herpetological Review 28 (3): 131.

Schick S., Kielgast J., Rödder D., Muchai V., Burger M. & Lötters S. (2010) New species of reed frog from the Congo basin with discussion of paraphyly in cinnamon-belly reed frogs. – Zootaxa 2501: 23–36.

Schiøtz A. (1975) The treefrogs of Eastern Africa. – Steenstrupia: Copenhagen.

Schiøtz A. (1999) Treefrogs of Africa. – Edition Chimaira: Frankfurt am Main.

Schiøtz A. (2006a) Notes on the genus *Hyperolius* (Anura, Hyperoliidae) in central République Démocratique du Congo. – Alytes 24: 40–60.

Schiøtz A. (2006b) Reflections on the *Hyperolius nasutus* group. – Alytes 24: 61–71.

Schiøtz A. (2008) *Hyperolius nasicus*. In: IUCN (2009) IUCN Red List of Threatened Species. Version 2009.1. <www.iucnredlist.org>. Downloaded on 9 January 2012.

Schiøtz A. & Drewes R. C. (2004) *Hyperolius discodactylus*. In: IUCN (2013) IUCN Red List of Threatened Species. Version 2013.1. Available from: http://www.iucnredlist.org/details/56130/0 (accessed on 26 August 2013).

Schiøtz A. & Poynton J. (2008) *Hyperolius benguellensis*. In: IUCN (2008) 2008 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 9 January 2012.

Schiøtz A. & van Daele P. (2003) Notes on the treefrogs (Hyperoliidae) of North-Western Province, Zambia. – Alytes 20: 137–149.

Schiøtz A., Channing A., Burger M. & Largen M. (2008a) *Hyperolius nasutus*. In: IUCN (2008) 2008 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 9 January 2012.

Schiøtz A., Channing A., Largen M., Howell K. & Lötters S. (2008b) *Hyperolius acuticeps*. In: IUCN (2008) 2008 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 9 January 2012.

Schmidt K. P. & Inger R. F. (1959) Amphibians exclusive of the genera *Afrixalus* and *Hyperolius*. – Exploration du Parc National de l'Upemba. Mission G.F. de Witte 56: 1–264.

Schneider H. & Sinsch U. (2007) Contributions of bioacoustics to the taxonomy of Anura. pp. 2893-2932 in: Heatwole H. & Tyler M. J. (eds.) Amphibian Biology – 7. Phylogeny and Systematics. Shipping Norton, Surrey Beatty & Sons.

Schoener T. W. (1974) Resource partitioning in ecological communities. – Science 185: 27–39.

Schoener T. W. (1986) Resource partitioning. pp. 91-126 in: Kikkawa J. & Anderson D. J. (eds.) Community Ecology: Community ecology: Pattern and process. Blackwell Scientific: Oxford.

Schubotz H. (1913) Wissenschaftliche Ergebnisse der Deutschen Zentral-Afrika-Expedition 1907-1908 unter Führung Adolf Friedrichs, Herzog zu Mecklenburg. Band III Zoologie I. – Klinkhardt und Biermann: Leipzig; 485 pp.

Seetzen U. J. (1855) Reisen durch Syrien, Palästina, Phönicien, die Transjordan-Länder, Arabia Petraea und Unter-Aegypten. Dritter Band. – G. Reimer: Berlin.

Sinsch U., Greenbaum E., Kusamba C. & Lehr E. (2011) Rapid assessment of montane anuran communities in the Albertine Rift: *Hyperolius castaneus* Ahl, 1931 as an umbrella species for conservation. – African Zoology 46: 320–333.

Sinsch U., Lümkemann K., Rosar K., Schwarz C. & Dehling J. M. (2012) Acoustic niche partitioning in an anuran community inhabiting an Afromontane wetland (Butare, Rwanda). – African Zoology 47: 60–73.

Smith A. (1849) Illustrations of the zoology of South Africa; consisting chiefly of figures and descriptions of the objects of natural history collected during an expedition into the interior of South Africa, in the years 1834, 1835, and 1836; fitted out by "The Cape of Good Hope association for exploring Central Africa:" together with a summary of African zoology, and an inquiry into the geographical ranges of species in that quarter of the globe Vol. III. Reptilia, Part 28. – Smith, Elder and Co.: Cornhill, London.

Spawls S., Howell K., Drewes R. & Ashe J. (2004) A field guide to the reptiles of East Africa [Reprint with corrections of the 2002 edition]. – A & C Black: London; 543 pp.

Spawls S., Howell K. M. & Drewes R. C. (2006) Pocket guide to the reptiles and amphibians of East Africa. – A & C Publishers: London.

Steindachner F. (1867) Reise der österreichischen Fregatte Novara um die Erde in den Jahren 1857,

1858, 1859 unter den Befehlen des Commodore B. von Wüllerstorf-Urbair. Zoologischer Theil. Erster Band. Amphibien. – Kaiserlich-Königliche Hof- und Staatsdruckerei: Wien.

Stewart M. M. (1985) Arboreal habitat use and parachuting by a subtropical forest frog. – Journal of Herpetology 19: 391–401.

Stewart M. M. & Halloy M. (2002) Raymond Laurent. – Copeia 2002: 245–247.

Storch V., Welsch U. & Wink M. (2007) Evolutionsbiologie. Zweite Auflage. – Springer: Berlin, Heidelberg.

Stuart S. N., Hoffmann M., Chanson J. S., Cox, N. A., Berridge R. J., Ramani P. & Young B. E. (2008) Threatened Amphibians of the World. – Lynx Edicions: Barcelona, Spain; IUCN: Gland, Switzerland; and Conservation International: Arlington, Virginia, USA.

Tawfik A. A., Akef M. S. A., Abdel-Mageid S. S. (1994) Electrophoretic and morphometric evidence for two species of the genus *Rana* (Amphibia, Ranidae) and some aspects of their variabilities. – Comparative Biochemistry and Physiology 107: 573–577.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. – Molecular Biology and Evolution 28: 2731–2739.

Templeton A. R., Crandall K. A. & Sing C. F. (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping aqnd DNA sequence data. III. Cladogram estimation. – Genetics 132: 619–633.

Tilman D. (2004) Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. – Proceedings of the National Academy of Sciences of the United States of America 10: 10854–10861.

Tobias M. L., Barnard C., O'Hagan R., Hornig S. H., Rand M. & Kelley D. B. (2004) Vocal communication between male *Xenopus laevis*. – Animal Behaviour 67: 353–365.

Toft C. A. (1985) Resource partitioning in amphibians and reptiles. – Copeia 1985: 1–21.

Toft C. A. & Duellman W. E. (1979) Anurans of the lower Rio Llullapichis, Amazonian Peru: a preliminary analysis of community structure. – Herpetologica 35: 71–77.

Tolley K. A., de Villers A. L., Cherry M. I. & Measey J. G. (2010) Isolation and high genetic diversity in dwarf mountain toads (*Capensibufo*) from South Africa. – Biological Journal of the Linnean Society 100: 822–834.

United Nations, Department of Economic and Social Affairs, Population Division (2011) World Population Prospects: The 2010 Revision, CD-ROM Edition.

van den Elzen P. & Kreulen D. A. (1979) Notes on the vocalisations of some amphibians from the Serengeti National Park, Tanzania. – Bonner zoologische Beiträge 30: 385–403.

Veith M., Kosuch J., Rödel M.-O., Hillers A., Schmitz A., Burger M. & Lötters S. (2009) Multiple evolution of sexual dichromatism in African reed frogs. – Molecular Phylogenetics & Evolution 51: 388–393.

Venables W. N. & Ripley B. D. (2002) Modern applied statistics with S. Fourth edition. – Springer: New York, NY, USA; 497 pp.

Vences M. & Wake D. B. (2007) Speciation, species boundaries and phylogeography of amphibians. pp. 2613–2671 in: Heatwole H. & Tyler M. J. (eds.) Amphibian Biology. – Surrey Beatty & Sons: Chipping Norton.

Vences M., Kosuch J., Glaw F., Böhme W. & Veith M. (2003) Molecular phylogeny of hyperoliid treefrogs: biogeographic origin of Malagasy and Seychellean taxa and re-analysis of familial paraphyly. – Journal of Zoological Systematics and Evolutionary Research 41 (3): 205–215.

Vences M., Kosuch J., Rödel M.-O., Lötters S., Channing A., Glaw F. & Böhme W. (2004) Phylogeography of *Ptychadena mascareniensis* suggests transoceanic dispersal in a widespread African-Malagasy frog lineage. – Journal of Biogeography 31: 593–601.

Vences M., Thomas M., van der Meijden A., Chiari Y. & Vieites D. R. (2005) Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. – Frontiers in Zoology 2: 1–12.

Vieites D. R., Wollenberg K. C., Andreone F., Köhler J., Glaw F. & Vences M. (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. – Proceedings of the National Academy of Science of the United States of America 196: 8267–8272.

Vonesh J. R. (2005) Egg predation and predator-induced hatching plasticity in the African reed frog, *Hyperolius spinigularis*. – Oikos 110 (2): 241–252.

Wägele H., Klussmann-Kolb A., Kuhlmann M., Haszprunar G., Lindberg D., Koch A. & Wägele J. W. (2011) The taxonomist - an endangered race. A practical proposal for its survival. – Frontiers in Zoology 8: 25.

Wager V. A. (1986) Frogs of South Africa, 2nd ed. – Delta Books: Craighall.

Wells K. D. (2007) The ecology and behavior of amphibians. – University of Chicago Press: Chicago.

Werner F. (1908, "1907") Ergebnisse der mit Subvention aus der Erbschaft Treitl unternommenen zoologischen Forschungsreise Dr. Franz Werner's nach dem ägyptischen Sudan und Nord-Uganda. XII. Die Reptilien und Amphibien. – Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Klasse 116: 1823–1926.

Wieczorek A. M., Drewes R. C. & Channing A. (2000) Biogeography and evolutionary history of *Hyperolius* species: application of molecular phylogeny. – Journal of Biogeography 27: 1231–1243.

Wieczorek A. M., Drewes R. C. & Channing A. (2001) Phylogenetic relationships within the *Hyperolius viridiflavus* complex (Anura: Hyperoliidae), and comments on taxonomic status. – Amphibia-Reptilia 22: 155–166.

World Factbook, The (2013/14) Central Intelligence Agency, 2013: Washington, DC. https://www.cia.gov/library/publications/the-world-factbook/index.html

Zimkus B. M. & Schick S. (2010) Light at the end of the tunnel: insights into the molecular systematics of East African puddle frogs (Anura: Phrynobatrachidae). – Systematics and Biodiversity 8: 39–47.

Zimkus B. M., Rödel M. O. & Hillers A. (2010) Complex patterns of continental speciation: Molecular phylogenetics and biogeography of sub-Saharan puddle frogs (*Phrynobatrachus*). – Molecular Phylogenetics & Evolution 55: 883–900.

Appendix

Appendices to Chapter III

Appendix 1. Material examined for morphological analyses. The mitochondrial 16S rRNA gene has been sequenced partially from the specimens marked with *.

Species	Sex	voucher #	origin	Species	sex	voucher #	origin
Ptychadena anchietae	male	ZFMK 94575*	Butare, Rwanda	*Ptychadena nilotica*	female	ZFMK 94604	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94576*	Butare, Rwanda	*Ptychadena nilotica*	female	ZFMK 94605	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94577	Butare, Rwanda	*Ptychadena nilotica*	female	ZFMK 94606	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94578	Butare, Rwanda	*Ptychadena nilotica*	female	ZFMK 94607	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94579	Butare, Rwanda	*Ptychadena nilotica*	female	ZFMK 94608	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94580	Butare, Rwanda	*Ptychadena nilotica*	female	ZFMK 94609	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94581	Butare, Rwanda	*Ptychadena nilotica*	female	ZFMK 94610	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94582	Butare, Rwanda	*Ptychadena nilotica*	female	ZFMK 94611	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94583	Butare, Rwanda	*Ptychadena nilotica*	female	ZFMK 94612	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94584	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94613*	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94585	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94614*	Butare, Rwanda
Ptychadena anchietae	male	ZFMK 94586	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94615*	Butare, Rwanda
Ptychadena anchietae	female	ZFMK 94587*	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94616	Butare, Rwanda
Ptychadena anchietae	female	ZFMK 94588	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94617	Butare, Rwanda

Species	Sex	voucher #	origin	Species	sex	voucher #	origin
Ptychadena anchietae	female	ZFMK 94589	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94618	Butare, Rwanda
Ptychadena nilotica	male	ZFMK 94590*	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94619	Butare, Rwanda
Ptychadena nilotica	male	ZFMK 94591*	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94620	Butare, Rwanda
Ptychadena nilotica	male	ZFMK 94592*	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94621	Butare, Rwanda
Ptychadena nilotica	male	ZFMK 94593*	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94622	Butare, Rwanda
Ptychadena nilotica	male	ZFMK 94594*	Butare, Rwanda	*Ptychadena porosissima*	male	ZFMK 94623	Butare, Rwanda
Ptychadena nilotica	male	ZFMK 94595	Butare, Rwanda	*Ptychadena porosissima*	female	ZFMK 94624*	Butare, Rwanda
Ptychadena nilotica	male	ZFMK 94596	Butare, Rwanda	*Ptychadena porosissima*	female	RMCA 109038	Tare, reg. Astrida
Ptychadena nilotica	male	ZFMK 94597	Butare, Rwanda	*Ptychadena porosissima*	female	RMCA 109085-095	Astrida, alt. 1750 m
Ptychadena nilotica	male	ZFMK 94598	Butare, Rwanda	*Ptychadena porosissima*	female	RMCA 109085-095	Astrida, alt. 1750 m
Ptychadena nilotica	male	ZFMK 94599	Butare, Rwanda	*Ptychadena porosissima*	female	RMCA 109085-095	Astrida, alt. 1750 m
Ptychadena nilotica	male	ZFMK 94600	Butare, Rwanda	*Ptychadena porosissima*	female	RMCA 109085-095	Astrida, alt. 1750 m
Ptychadena nilotica	male	ZFMK 94601	Butare, Rwanda	*Ptychadena porosissima*	female	RMCA 109039	Tare, Busanza
Ptychadena nilotica	male	ZFMK 94602	Butare, Rwanda	*Ptychadena porosissima*	female	RMCA 109051-056	Karambi, Terr. De Nyanza
Ptychadena nilotica	female	ZFMK 77757*	Cairo, Egypt	*Ptychadena porosissima*	female	RMCA 109051-056	Karambi, Terr. De Nyanza
Ptychadena nilotica	female	ZFMK 94603*	Butare, Rwanda				

Appendix 2. List of samples used for analysis, their geographic origin, voucher specimens and Genbank accession numbers

Species	Origin	Voucher	GenBank #
P. aequiplicata	Ghana: Wli Waterfalls	MOR G56	AY517613
P. aff. *aequiplicata*	Cameroon: Dja Reserve	CAS199182	DQ525919
P. anchietae	Kenya: Runda	NMK A_3849_1	AY517611
P. anchietae	Kenya: Runda	"SLcoll"	AY517612
P. anchietae	Kenya: Kararacha Pond	CAS214837	DQ525920
P. anchietae	Kenya:_Kakamega Forest	NMK A_3845	AY517609
P. anchietae	Rwanda: Butare	ZFMK 94575–76	KF027213
P. anchietae	Somalia: Karin	CAS227507	DQ525922
P. anchietae	Somalia: Karin	CAS227562	DQ525921
P. anchietae	South Africa: Mtunzini	Not collected	AF215404
P. anchietae	South Africa: St. Lucia	Not collected	AF215405
P. anchietae	Tanzania: Makuyuni	SL1_02	AY517610
P. anchietae	Uganda: Semliki NP	SL528	GQ183596
P. anchietae	Uganda: Semliki NP	SL523	GQ183597
P. anchietae	Uganda: Semliki NP	SL529	GQ183598
P. anchietae	Not provided	VUB 0958	AY948742
P. bibroni	Ivory Coast: Mont Sangbé National Park	Not collected	AY517602
P. bibroni	Ivory Coast: Taï National Park	MOR T38	AY517603
P. aff. *bibroni*	Gabon: Monts Cristal, Tchimbéle	SL1036	AY517604
P. longirostris	Ivory Coast: Mont Sangbé National Park	S01_34	AY517606
P. mahnerti	Kenya: Mt Kenya	SL171	DQ525918
P. mascareniensis	Madagascar: Antsiranana, Ambanja, Mandrizavona Village, Ramena Valley, 13°48′3 S, 48°44′47 E	AMNH A167415	DQ283031
P. mascareniensis	Madagascar: Betampona	MRSN:A6351	HM364770
P. mascareniensis	Madagascar: Itremo	FAZC 14045	JF903872
P. mascareniensis m1	Madagascar: Nahampoana	ZSM 190_2002	AY517587
P. mascareniensis m2	Madagascar: Andring	ZSM 717_2001	AY517588
P. mascareniensis m3	Mauritius	ZSM 984_2000	AY517589
P. mascareniensis m4	Madagascar: Fierenana	ZSM 252_2002	AY517590
P. mascareniensis m5	Madagascar: Ambanja	ZSM 421_2000	AY517591
P. mascareniensis m6	Madagascar: NosyBe	UADBA 2001_02	AY517592
P. mascareniensis m7	Madagascar: Mantasoa	UADBA 2000_04	AY517593
P. mascareniensis m8	Madagascar: Sambava	ZSM 562_2000	AY517594
P. aff. *mascareniensis* B	Benin: Lama forest	ZFMK 77100	AY517597
P. aff. *mascareniensis* B	Cameroon	ZFMK 68826	AF215408
P. aff. *mascareniensis* C	Ivory Coast: Mont Sangbé National Park	MOR S01_40	AY517598
P. aff. *mascareniensis* D	Uganda: Kampala	MVZ234084	DQ525931
P. aff. *mascareniensis* D	Uganda: Rwenzori Mts., Nyakalengija	SL479	GQ183592
P. aff. *mascareniensis* D	Uganda: Rwenzori Mts., Nyakalengija	SL463	GQ183593
P. aff. *mascareniensis* D	Kenya: Kakamega Forest	NMK A_3840_1	AY517599
P. aff. *mascareniensis* E	Central African Republic: Dzanga-Sangha Reserve	DS 52	DQ525932
P. newtoni	Sao Tome and Principe: Sao Tome	CAS 21950	GU457591
P. newtoni	Sao Tome and Principe	CAS 219251	DQ525935
P. newtoni	Sao Tome and Principe	CAS219252	DQ525936
P. newtoni	Sao Tome and Principe	CAS219250	DQ525934
P. newtoni	Sao Tome and Principe	CAS219253	DQ525937
P. nilotica	Uganda: Lake Victoria	MVZ234085	DQ525923
P. nilotica	Kenya: Mt Kenya	MVZ234086	DQ525926
P. nilotica	Kenya: Mt Kenya	MVZ234087	DQ525925
P. nilotica	Egypt: Cairo	ZFMK 77757	AY517596
P. nilotica	Kenya: "Makuru" [= Nakuru]	MVZ223624	DQ525924
P. nilotica	Tanzania: Kibebe Farm	AC1728	AY517595
P. nilotica	Tanzania: Kibebe Farm, Iringa	AC2087	DQ525929
P. nilotica	Kenya: Taita Hills	CAS191517	DQ525927
P. nilotica	Kenya: Taita Hills	CAS191518	DQ525928
P. nilotica	Uganda: Semliki NP	SL533	GQ183594
P. nilotica	Rwanda: Butare	ZFMK 94591, 94593, 94594	KF027211
P. nilotica	Rwanda: Butare	ZFMK 94592	KF027214
P. oxyrhynchus	Namibia: Rundu	Not collected	AF215409
P. oxyrhynchus	Malawi	Malawi359	DQ525939
P. porosissima	Rwanda: Butare	ZFMK 94613–14	KF027212
P. porosissima	South Africa: Kwambonambi	Not collected	AF215411
P. porosissima	Tanzania: Mumba	AC2122	AY517601
P. porosissima	Tanzania: Mumba	AC 2122	DQ525941
P. aff. *porosissima*	Tanzania: Tatanda	AC2034	DQ525940
P. pumilio	Ghana	MOR G79	AY517600
P. schubotzi	Ivory Coast	IvCoast_S01_22	AY517607
P. aff. *schubotzi*	Kenya: Kakamega	SL042 (tadpole)	AY517608
P. taenioscelis	Kenya: Kakamega	NMKA3955	DQ525943
P. aff. *uzungwensis*	Tanzania: Njombe	AC1970	DQ525945
Hildebrandtia ornata	Ivory Coast: Comoé National Park	Not collected	AF215402

Appendix 3. Description of a topotypic specimen of *Ptychadena nilotica* (Seetzen, 1855) from Cairo, Egypt

Topotype: ZFMK 77757, subadult female, from Gabala/Fayoum, Cairo, Egypt.

Genetic information: Partial sequence (532 bp) of the mitochondrial 16S rRNA gene, GenBank Accession Number AY517596 (Vences *et al.* 2004).

Description: Body moderately sturdy, widest at temporal region, slightly tapering to groin; head large (HL/SVL 0.34; HW/SVL 0.33), longer than wide (HL/HW 1.19); snout long (SL/HL 0.43), pointed in dorsal view, rounded in profile, considerably projecting beyond lower jaw, longer than wide (SL/EE 1.36); canthus rostralis distinct between eye and nostril, straight-lined; loreal region oblique, moderately concave; nostrils rounded, directed dorsolaterally; situated half-way between tip of snout and eye (EN/NS 0.98), separated from each other by distance slightly less than distance between eye and nostril (NN/EN 0.91); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.28), its diameter shorter than snout (ED/SL 0.65); interorbital distance equalling upper eyelid width (IO/EW 1.03), smaller than internarial distance (IO/NN 0.74); tympanum and its annulus distinctly visible, separated from eye by about two-fifths of its diameter (ET/TD 0.42); tympanum diameter smaller than eye diameter (TD/ED 0.82); upper jaw with dentition; choanae small, rounded, located far anterolaterally at margins of roof of the mouth; vomer teeth in two short rows, situated at medial edges of choanae, separated from each other by distance about three times the length of individual row; tongue long and narrow, bilobed for about one-sixth of its length, free distally for one-third its length; median lingual process absent.

Dorsal surfaces of head, trunk and limbs shagreened; dorsum with four longitudinal dermal ridges on each side, median ones extending from infraorbital region, lateral ones from level just behind posterior edge of upper eyelid to vent; lateralmost fold very prominent, extending from just behind posterior corner as dorsolateral fold to groin; ventral side of limbs and body smooth except areolate postaxial side of thigh; distinct transverse fold between arms on ventral side.

Forelimbs moderately sturdy; hand relatively small (HND/SVL 0.26); tips of fingers rounded, not enlarged into disks but slightly swollen volarly; transverse dorsal skin ridge separating the ultimate from the other phalanges on each finger; relative length of fingers: I≤II < IV< III; subarticular tubercles rounded, well developed, numbering one on Fingers I and II, two on Fingers III and IV; proximal tubercles on Fingers III and IV larger and more prominent than distal ones; finger webbing absent; thenar tubercle prominent, elongated and narrow, comparatively small, less than half the length of base of thumb; inner palmar tubercle small, flat, less conspicuous, on proximal one-third of metacarpal region of Fingers II and III, rounded, flat; outer palmar tubercle on proximal half of metacarpal region of Finger IV, prominent, elongate and narrow; single supernumerary metacarpal tubercle between outer palmar tubercle and proximal subarticular tubercle of Finger IV; callous longitudinal ridges between subarticular tubercles on Fingers III and IV and between subarticular tubercles and finger tips on all fingers.

Hindlimbs sturdy, long (LEG/SVL 1.82); tibiofibula moderately long (TFL/SVL 0.60), longer than thigh (TFL/THL 1.12); heels overlapping each other when knees flexed and thighs held perpendicularly to median plane; two low longitudinal ridges on tarsus between heel and metatarsal tubercles; foot long, longer than tibiofibula (FOT/TFL 1.12); relative length of toes: I < II < III<V<IV; Toes IV and V of left foot removed as tissue sample; toe tips rounded, not enlarged into disks but slightly swollen plantarly; transverse dorsal skin ridge separating the ultimate from the other phalanges on each toe; subarticular tubercles numbering one on Toes I and II, two on Toes III and V, and three on Toe IV; low callous ridges between metatarsus and subarticular tubercles, between subarticular tubercles, and between subarticular tubercles and toe tips; pedal webbing formula I1.5-2II1.25-2.5III1.5-3IV2.75-1V; inner metatarsal tubercle elongated, prominent, small, about half the length of phalanges of Toe I; outer metatarsal tubercle rounded, flat, distinctly present.

Measurements [in mm]: SVL 36.9, TFL 22.2, THL 15.8, LEG 67.4, TarL 32.8, FOT 23.3, HND 9.70, HW 12.2, HL 14.6, IO 2.2, EW 2.1, ED 4.1, TD 3.4, EN 3.2, NS 3.3, SL 6.3, and NN 2.9.

Appendices to Chapter IV

Appendix 4. Material examined.

Ptychadena anchietae: Butare [= Huye], Rwanda (ZFMK 94575–89; twelve males, three females); "Kisenyi (Kivu)" [= Gisenyi/Rubavu, Rwanda] (RMCA 51565–67; three males).

Ptychadena chrysogaster: „Lac Karago, alt. 2250 m, terr. De Kisenyi", Rwanda (RMCA 109096, one female, holotype; RMCA 109097–109113, sixteen males, paratypes); "Lulenga, Kivu", DRC (RMCA 3452–69, eleven males, one female, paratypes; 2518–2521, four males, paratypes; 2759–68, one female, nine males, paratypes; 1748–55, seven males, one female, paratypes); "Gatsibu", Rwanda (RMCA 36844, one male, paratype; 36866, one male, paratype); "Ruhengeri, riv. Moklungwa, alt. 1800–1825 m.), Ruanda" (RMCA 42011–13, three females, paratypes); "Lac Gando, alt. 2400 m, Ruanda" (RMCA 42025–28, four males, paratypes); "Region de Mulera, alt. 1800–2000 m", Rwanda (RMCA 41987–88, one male, one female, paratypes); "Kasenze (versant S. Karisimbi)", Rwanda (RMCA 42022–23, two males, paratypes); "entre Managna et Tshengelero, alt. 1750–2000 m", DRC (RMCA 41965–41970, four males, one female, paratypes); "Kundhuru-Tshuve (col. Gahinga-Sabinyo), alt. 2600 m", Rwanda (RMCA 41982–83, one male, one female, paratypes; RMCA 41984–86, three males, paratypes); "Ruhengeri, sources Kirii, alt. 1800–1825 m", Rwanda (RMCA 41991, female, 41992–93, two males, paratypes); "Riv. Rodahira, afflt. de la riv. Fuku, s/afflt. de la riv. Rutshuru, près de Rutshuru, alt. 1250 m", DRC (RMCA 116959, one male); "Nyabitsindi, entre le Visoke et le Musule, alt. 2400 m", Rwanda (RMCA 42016–17, one male, one female, paratypes); "Kibga, riv. Suza, versant Sud Visoke, alt. 2400 m", Rwanda (RMCA 42018, one male, paratype); "Dubi versant S. Visoke, Ruanda" (RMCA 42019–21, one female, two males, paratypes); "Shamuheru, Nyamuragira, alt. 1843 m", DRC (RMCA 42031–32, one male, one female, paratypes); "Munagana, marais de Maziba, alt 2000 m", Uganda (RMCA 41979–80, one male, one juvenile, paratypes); "Ilega (versant S. Karisimbi), Rubinda, alt. 2400 m", Rwanda (RMCA 42024, subadult, paratype); "Kagogo, Lac Bulera, terr. de Ruhengeri, alt. 1870 m (Ruanda)" (RMCA 109121, one female; 109122, one male, paratypes); "Remera, Lac Luhondo, alt. 1770 m, terr. de Ruhengeri (Ruanda)" (RMCA 109114–120, three males, four females, paratypes); "Mutabonika, près de Ngabitsindi entre le Visoke et le Musule, alt. 2400 m" (RMCA 42014, one female; 42015, one male; paratypes); "Bitare, alt. 1650 m, Terr. de Kitega, Urundi" [= Burundi] (RMCA 109161–62, two males, paratypes); "Vyuya, Terr. de Bururi, alt. 1900 m (Urundi)" [=Burundi] (RMCA 109163–66, two males, paratypes); "Astrida, alt. 1750 m. (Ruanda)" [=Butare/Huye, Rwanda] (RMCA 109139–141, two females, one male, paratypes); "Tare, Busanza, région d'Astrida, alt. 1700 m (Ruanda)" (RMCA 109142, one female; 109143–147, four males, one female, paratypes); Mugatemba, Pref. Gikongoro, Rwanda (ZFMK 58747, one female); Nyungwe-Wald, Mukina (Kitabi), Rwanda (ZFMK 58797, one

male); Cyamudongo, (ZFMK 58847–850); Nyakalengijo, Mt Ruwenzori, Uganda (ZFMK 63239–241).

Ptychadena grandisonae: "Muita-Luembe, E (Angola)" (RMCA 60530, one female, holotype); ""Bitare, alt. 1650 m, Terr. de Kitega, Urundi" [= Burundi] (RMCA 109036–37, one male, one female, paratypes).

Ptychadena guibei: "Muita-Luembe, E (Angola)" (RMCA 60535, female, holotype).

Ptychadena nilotica: Butare, Rwanda (ZFMK 94590–612, thirteen males, ten females; JMD 807, one male; EL 15-17, 25-26, 37, one male, five females); Mashyuza, Nyakabuye, Rwanda (ZFMK 58839–846); Route Kigali – Byumba, Rwanda (ZFMK 58851–852); Cyangugu, Rwanda (JMD 961, one male); Lac Karago, Rwanda (JMD 1028-1029); Bugarama, Rwanda (JMD 1074, one male); Bururi, Burundi (JMD 1001-1002, two males); Ruzizi National Park, Burundi (JMD 1015-1016, one male, one female). [JMD& EL specs currently being assigned ZFMK nos.]

Ptychadena porosissima: Butare [= Huye], Rwanda (ZFMK 94613–24, eleven males, one female); "Tare, reg. Astrida", Rwanda (RMCA 109038, one female, holotype of *Ptychadena loveridgei* Laurent, 1954); "Astrida, alt. 1750 m" [= Huye], Rwanda (RMCA 109085–095, seven males, four females, paratypes of *Ptychadena loveridgei*); "Tare, Busanza", Rwanda (RMCA 109039, one female, paratype of *Ptychadena loveridgei*); "Karambi, Terr. De Nyanza", Rwanda (RMCA 109051–056, two females, paratypes of *Ptychadena loveridgei*); "Ruhengeri, riv. Moklungwa, alt. 1800–1825 m., Ruanda" (RMCA 42003–10, eight males, paratypes of *Ptychadena loveridgei*); "Ruhengeri, sources Kirii, alt. 1800–1825 m", Rwanda (RMCA 41994, paratype of *P. chrysogaster*); "Reg. du Rwankeri, alt. 2200 m, Ruanda" (RMCA 41989, one male, paratype of *P. chrysogaster*).

Ptychadena taenioscelis: "Lukulu près Kiambi", DRC (RMCA 13122, one subadult female, holotype).

Ptychadena uzungwensis: „Dabaga, Utschungwe Mts.", Tanzania (RMCA 58843, one male, paratype); "Kumunini, Buyenza, alt. 2000 m, terr. d' Astrida (Ruanda)" [= Munini, Rwanda] (RMCA 108993–97, five males); "Astrida, marais de la Mukura, alt. 1700 m (Ruanda)" [= Huye, Rwanda] (RMCA 108992, one female); "Bitare, alt. 1650 m, Terr. de Kitega, Urundi" (RMCA 108999–109016).

Appendix 5. Morphological features of *Ptychadena* spp. from Rwanda

The external morphology of each species is described in the following account. Descriptions are primarily based on Rwandan material. In case of *P. chrysogaster* we also included data from type specimens from Burundi, Uganda, and the Democratic Republic of the Congo. The description of *P. uzungwensis* is based on all available material from Rwanda and a male paratype from the type locality (see Appendix 4).

Ptychadena anchietae

Body moderately sturdy, widest at temporal region, slightly tapering to groin; head large (HL/SUL 0.36–0.43 in males, 0.37–0.41 in females; HW/SUL 0.31–0.39 in males, 0.33–0.36 in females), longer than wide (HL/HW 1.01–1.26 in males, 1.11–1.13 in females); snout long (SL/HL 0.43–0.53 in males, 0.49–0.53 in females), pointed in dorsal view, rounded in profile, considerably projecting beyond lower jaw, longer than wide (SL/EE 1.08–1.24 in males, 1.30–1.33 in females); canthus rostralis distinct between eye and nostril, straight-lined; loreal region oblique, strongly concave; nostrils rounded, directed dorsolaterally; situated half-way between tip of snout and eye or closer to tip of snout than to eye (EN/NS 0.98–1.33 in males, 1.14–1.27 in females), separated from each other by distance subequal to distance between eye and nostril (NN/EN 0.87–1.05 in males, 0.85–0.96 in females); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.24–0.29 in males, 0.26–0.29 in females), its diameter much shorter than snout (ED/SL 0.50–0.63 in males, 0.53–0.58 in females); interorbital distance more or less equalling upper eyelid width (IO/EW 0.83–1.10 in males, 0.89–1.06 in females) and smaller than internarial distance (IO/NN 0.59–0.83 in males, 0.63–0.67 in females); tympanum and its annulus distinctly visible, separated from eye by about one-fifth to one-third of its diameter (ET/TD 0.21–0.36 in males, 0.19–0.25 in females); tympanum diameter 0.62–0.92 (in males) and 0.74–0.83 (in females) of eye diameter; upper jaw with dentition; choanae small, rounded, located far anterolaterally at margins of roof of mouth; vomer teeth in two short rows, separated from each other by distance about three times length of individual row; tongue long and narrow, bilobed for about one-sixth of its length, free distally for one-fourths its length; median lingual process absent; vocal sac in males paired, lateral; external vocal sac aperture as a longitudinal, posterolaterally orientated slit, inferior, terminating at level of ventral edge of insertion of arms; internal vocal sac apertures rounded, situated close to corner of mouth.

Dorsal surfaces of head, trunk and limbs finely shagreened with many scattered small tubercles; dorsum with five or six longitudinal dermal ridges on each side; median ridge extending from interorbital region almost to vent, postpalpebral and external ridges from level just behind posterior edge of upper eyelid to insertion of leg; laterodorsal ridge extending from level about one snout length posterior to tympanum to groin; dorsal ridges interrupted in few specimens; sacral ridge extending from about one head length anterior to vent either medially to median ridges or between median and postpalpebral ridges to vent, in few specimens absent; external ridge forming anterior part of supratympanic fold; posterior part of supratympanic fold less distinct, branching off from external dorsal ridge posterior to tympanum in wide angle and extending posterolaterally to insertion of arm; infratympanic fold thick and conspicuous, almost straight-lined, extending from ventral edge of eye to level of arm insertion, meeting with supratympanic fold; ventral side of limbs and body smooth except slightly areolate postaxial side of thigh; distinct transverse fold between arms on ventral side; supratympanic fold moderately distinct, angled, extending

from posterior corner of eye to point dorsal from arm insertion; infratympanic fold thick and conspicuous, almost straight-lined, extending from ventral edge of eye to level of arm insertion, meeting with supratympanic fold, continued after a small gap in form of large oval tubercle dorsally of posterior end of arm insertion.

Forelimbs moderately sturdy; hand relatively small (HND/SUL 0.23–0.27 in males, 0.25–0.26 in females); tips of fingers rounded, not enlarged into disks but slightly swollen volarly; transverse dorsal skin ridge separating ultimate from other phalanges on each finger; relative length of fingers: I ≤ II < IV < III; subarticular tubercles rounded, well developed, numbering one on Fingers I and II, two on Fingers III and IV, proximal tubercles on Fingers III and IV larger and more prominent than distal ones; finger webbing absent; thenar tubercle distinct, large, flat, oval, slightly more than half as long as metacarpal of Finger I; inner palmar tubercle on proximal half of metacarpal region of Fingers II and III, rounded, flat; outer palmar tubercle on proximal half of metacarpal region of Finger IV, oval, slightly more prominent than inner palmar tubercle; one supernumerary metacarpal tubercle between palmar or thenar tubercles and proximal subarticular tubercles on each finger; callous longitudinal ridges between subarticular tubercles on Fingers III and IV and between subarticular tubercles and finger tips on all fingers; nuptial pads in males covering almost entire dorsal surfaces of Fingers I and II, and proximal portion of dorsal side of Finger III.

Hindlimbs sturdy, very long (LEG/SUL 1.87–2.11 in males, 1.96–2.13 in females); knee reaching slightly beyond insertion of forelimbs and tibio-tarsal articulation reaching almost a head length beyond tip of snout when legs are adpressed forwardly to body; tibiofibula very long (TFL/SUL 0.61–0.68 in males, 0.65–0.72 in females), longer than thigh (TFL/THL 1.09–1.19 in males, 1.13–1.16 in females); heels overlapping each other considerably when knees flexed and thighs held perpendicularly to median plane; two low longitudinal ridges on plantar side of tarsus between heel and metatarsal tubercles; foot shorter than or equal in length to tibiofibula (FOT/TFL 0.85–1.00 in males, 0.91–0.93 in females); relative length of toes: I < II < III < V < IV; toe tips rounded, not enlarged into disks but slightly swollen plantarly; transverse dorsal skin ridge separating ultimate from other phalanges on each toe; subarticular tubercles numbering one on Toes I and II, two on Toes III and V, and three on Toe IV; low callous ridges between subarticular tubercles, and between subarticular tubercles and toe tips; pedal webbing formula I0.5-2II0.5-2III(0.5–1)-2IV2-0.5V; inner metatarsal tubercle moderately large, half as long as metatarsus of Toe I, oval, prominent; outer metatarsal tubercle rounded, flat, faintly visible, callous tissue weakly developed.

Ptychadena chrysogaster

Body moderately sturdy, widest at temporal region, slightly tapering to groin; head large (HL/SUL 0.34–0.38 in males, 0.33–0.37 in females; HW/SUL 0.29–0.34 in males, 0.28–0.34 in females), longer than wide (HL/HW 1.04–1.22 in males, 1.05–1.23 in females); snout long (SL/HL 0.45–0.50 in males, 0.45–0.51 in

females), pointed in dorsal view, rounded in profile, considerably projecting beyond lower jaw, longer than wide (SL/EE 1.11–1.28 in males, 1.11–1.37 in females); canthus rostralis distinct between eye and nostril, straight-lined; loreal region oblique, strongly concave; nostrils rounded, directed dorsolaterally; situated more or less half-way between tip of snout and eye (EN/NS 0.87–1.14 in males, 0.93–1.24 in females), separated from each other by distance subequal to or larger than distance between eye and nostril (NN/EN 1.02–1.22 in males, 0.94–1.24 in females); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.27–0.32 in males, 0.26–0.30 in females), its diameter much shorter than snout (ED/SL 0.57–0.66 in males, 0.51–0.66 in females); interorbital distance larger than upper eyelid width (IO/EW 1.12–1.48 in males, 1.09–1.44 in females) and smaller than internarial distance (IO/NN 0.75–0.89 in males, 0.78–0.91 in females); tympanum and its annulus distinctly visible, separated from eye by about one-fourth to two-fifths of its diameter (ET/TD 0.24–0.38 in males, 0.26–0.40 in females); tympanum diameter slightly smaller to subequal to eye diameter (TD/ED 0.76–0.98 in males, 0.85–1.01 in females); upper jaw with dentition; choanae small, rounded, located far anterolaterally at margins of roof of mouth; vomer teeth in two short rows, separated from each other by distance about three times length of individual row; tongue long and narrow, bilobed for about one-fifth of its length, free distally for one-fourth its length; median lingual process absent; vocal sac in males paired, lateral; external vocal sac aperture as a longitudinal, posterolaterally orientated slit, inferior, terminating at level of ventral edge of ventral insertion of arms; internal vocal sac apertures rounded, situated close to corner of mouth.

Dorsal surfaces of head, trunk and limbs finely shagreened; dorsum with five or six longitudinal dermal ridges on each side, median one extending from interorbital region almost to vent, postpalpebral and external ones from level just behind posterior edge of upper eyelid to insertion of leg; laterodorsal ridge extending from level about one snout length posterior to tympanum to groin; sacral ridge extending from about one head length anterior to vent either medially to median ridges or between median and postpalpebral ridges to vent; in few specimens additional sacromedial ridge extending between sacral ridge and median ridge to vent for about half length of sacral ridge; external ridge forming anterior part of supratympanic fold; posterior part of supratympanic fold less distinct, branching off from external dorsal ridge posterior to tympanum in wide angle and extending posterolaterally to insertion of arm; infratympanic fold thick and conspicuous, almost straight-lined, extending from ventral edge of eye to level of arm insertion, meeting with supratympanic fold; ventral side of limbs and body smooth except areolate proximal postaxial-ventral part of thigh; distinct transverse fold between arms on ventral side; ventral side of trunk and head densely covered with more or less evenly scattered tiny, pointed tubercles in males.

Forelimbs moderately sturdy; hand relatively small (HND/SUL 0.22–0.26 in males, 0.21–0.25 in females); tips of fingers rounded, not enlarged into disks but slightly swollen volarly; transverse dorsal skin ridge separating ultimate from other phalanges on each finger; relative length of fingers: I = II < IV < III;

subarticular tubercles rounded, well developed, numbering one on Fingers I and II, two on Fingers III and IV, proximal tubercles on Fingers III and IV larger and more prominent than distal ones; finger webbing absent; thenar tubercle prominent, elongated, large, two-thirds length of metacarpal of Finger I; inner palmar tubercle on proximal third of metacarpal region of Fingers II and III, oval, flat; outer palmar tubercle on proximal half of metacarpal region of Finger IV, elongated, slightly more prominent than inner palmar tubercle; one supernumerary metacarpal tubercle between palmar or thenar tubercles and proximal subarticular tubercles on all fingers; low callous longitudinal ridges between subarticular tubercles on Fingers III and IV and between subarticular tubercles and finger tips on all fingers; nuptial pads in males covering almost entire dorsal surfaces of Fingers I and II except distal phalanx, and preaxial half of dorsal side of metacarpal of Finger III.

Hindlimbs sturdy, very long (LEG/SUL 1.94–2.27 in males, 1.93–2.14 in females); knee reaching to insertion of forelimbs and tibio-tarsal articulation reaching slightly more than a snout length beyond tip of snout when legs adpressed forwardly to body; tibiofibula very long (TFL/SUL 0.61–0.73 in males, 0.62–0.70 in females), longer than thigh (TFL/THL 1.13–1.22 in males, 1.13–1.25 in females); heels overlapping each other considerably when knees flexed and thighs held perpendicularly to median body plane; two low longitudinalridges on plantar side of tarsus between heel and metatarsal tubercles; foot subequal in length to tibiofibula (FOT/TFL 0.97–1.07 in males, 0.94–1.03 in females); relative length of toes: I < II < III < V < IV; toe tips rounded, not enlarged into disks but slightly swollen plantarly; transverse dorsal skin ridge separating ultimate from other phalanges on each toe; subarticular tubercles numbering one on Toes I and II, two on Toes III and V, and three on Toe IV; low callous ridges between subarticular tubercles, and between subarticular tubercles and toe tips; pedal webbing formula I2-2.5II(1.5–1.75)-3III(2–2^{-})(3.25–3^{+})IV3-(1.5–2)V; inner metatarsal tubercle elongated, prominent, small, less than half as long as metatarsus of Toe I; outer metatarsal tubercle very faintly visible, rarely prominent, small and pointed.

Ptychadena nilotica

Body moderately sturdy, widest at temporal region, slightly tapering to groin; head large (HL/SUL 0.35–0.43 in males, 0.35–0.39 in females; HW/SUL 0.30–0.37 in males, 0.32–0.35 in females), longer than wide (HL/HW 1.10–1.27 in males, 1.06–1.15 in females); snout long (SL/HL 0.38–0.46 in males, 0.40–0.45 in females), pointed in dorsal view, rounded in profile, considerably projecting beyond lower jaw, longer than wide (SL/EE 1.20–1.40 in males, 1.15–1.36 in females); canthus rostralis distinct between eye and nostril, straight-lined; loreal region oblique, moderately concave; nostrils rounded, directed dorsolaterally; situated half-way between tip of snout and eye or closer to tip of snout than to eye (EN/NS 0.99–1.18 in males, 1.05–1.34 in females), separated from each other by distance slightly less than or subequal to distance between eye and nostril (NN/EN 0.82–0.99 in males, 0.87–0.97 in females); eyes directed anterolaterally,

moderately protruding, relatively small (ED/HL 0.25–0.32 in males, 0.26–0.29 in females), its diameter shorter than snout (ED/SL 0.60–0.69 in males, 0.59–0.69 in females); interorbital distance smaller to equaling upper eyelid width (IO/EW 0.64–1.03 in males, 0.72–0.90 in females) and smaller than internarial distance (IO/NN 0.55–0.74 in males, 0.57–0.72 in females); tympanum and its annulus distinctly visible, separated from eye by about one-fourth to one-third of its diameter (ET/TD 0.28–0.36 in males, 0.26–0.37 in females); tympanum diameter 0.75–0.88 (in males) and 0.77–0.89 (in females) of eye diameter; upper jaw with dentition; choanae small, rounded, located far anterolaterally at margins of roof of mouth; vomer teeth in two short rows, separated from each other by distance about three times length of individual row; tongue long and narrow, bilobed for about one-sixth of its length, free distally for one-third its length; median lingual process absent; vocal sac in males paired, lateral; external vocal sac aperture as a longitudinal, posteriorly orientated slit, situated dorsally of level of insertion of arms, parallel to mandible and infratympanic fold, covered by narrow dermal flap on ventral edge of slit; internal vocal sac apertures rounded, situated close to corner of mouth.

Dorsal surfaces of head, trunk and limbs shagreened; dorsum with four or five longitudinal dermal ridges on each side, median one extending from interorbital region almost to vent, postpalpebral and external ones from level just behind posterior edge of upper eyelid to insertion of leg; laterodorsal ridge extending from level about one snout length posterior to tympanum to groin; in few specimens additional very short ridge present between median and postpalpebral ridge from about one snout length anterior to vent extending almost to vent; external ridge forming anterior part of supratympanic fold; posterior part of supratympanic fold less distinct, branching off from external dorsal ridge posterior to tympanum in wide angle and extending posterolaterally to insertion of arm; infratympanic fold thick and conspicuous, almost straight-lined, extending from ventral edge of eye to level of arm insertion, meeting with supratympanic fold; ventral side of limbs and body smooth except areolate postaxial side of thigh; distinct transverse fold between arms on ventral side.

Forelimbs moderately sturdy; hand relatively small (HND/SUL 0.21–0.27 in males, 0.22–0.26 in females); tips of fingers rounded, not enlarged into disks but slightly swollen volarly; transverse dorsal skin ridge separating ultimate from other phalanges on each finger; relative length of fingers: I ≤ II < IV < III; subarticular tubercles rounded, well developed, numbering one on Fingers I and II, two on Fingers III and IV; proximal tubercles on Fingers III and IV larger and more prominent than distal ones; finger webbing absent; thenar tubercle prominent, elongated and narrow, comparatively small, less than half as long as metacarpal of Finger I; inner palmar tubercle small, flat, less conspicuous, on proximal one-third to one-fourth of metacarpal region of Fingers II and III, rounded, flat; outer palmar tubercle on proximal half of metacarpal region of Finger IV, prominent, elongate and narrow; single supernumerary metacarpal tubercle between outer palmar tubercle and proximal subarticular tubercle of Finger IV, often indistinct; callous

longitudinal ridges between subarticular tubercles on Fingers III and IV and between subarticular tubercles and finger tips on all fingers; nuptial pads in males covering almost entire dorsal surfaces of Fingers I and II and preaxial half of dorsal surface of Finger III.

Hindlimbs sturdy, long (LEG/SUL 1.71–1.97 in males, 1.76–1.96 in females); knee reaching just behind insertion of forelimbs and tibio-tarsal articulation reaching tip of snout when legs are adpressed forwardly to body; tibiofibula moderately long (TFL/SUL 0.50–0.60 in males, 0.54–0.60 in females), longer than thigh (TFL/THL 1.04–1.12 in males, 1.09–1.12 in females); heels overlapping each other when knees flexed and thighs held perpendicularly to median plane; two low longitudinal ridges on plantar side of tarsus between heel and metatarsal tubercles; foot long, longer than tibiofibula (FOT/TFL 1.07–1.14 in males, 1.03–1.10 in females); relative length of toes: I < II < III < V < IV; toe tips rounded, not enlarged into disks but slightly swollen plantarly; transverse dorsal skin ridge separating ultimate from other phalanges on each toe; subarticular tubercles numbering one on Toes I and II, two on Toes III and V, and three on Toe IV; low callous ridges between subarticular tubercles, and between subarticular tubercles and toe tips; pedal webbing formula I(1.5–1.75)-(2–2.25)II1.5-(2.75–3)III(1.75–2)-3IV2.75-(1–1.5)V; inner metatarsal tubercle elongated, prominent, small, less than half as long as metatarsus of Toe I; outer metatarsal tubercle rounded, flat, distinctly present, rarely faintly visible.

Ptychadena porosissima

Body moderately sturdy, widest at temporal region, slightly tapering to groin; head large (HL/SUL 0.35–0.41 in males, 0.35–0.40 in females; HW/SUL 0.31–0.36 in males, 0.30–0.34 in females), longer than wide (HL/HW 1.02–1.19 in males, 1.03–1.21 in females); snout moderately long (SL/HL 0.38–0.49 in males, 0.43–0.51 in females), pointed in dorsal view, rounded in profile, considerably projecting beyond lower jaw, longer than wide (SL/EE 1.21–1.27 in males, 1.11–1.61 in females); canthus rostralis distinct between eye and nostril, straight-lined; loreal region oblique, strongly concave; nostrils rounded, directed dorsolaterally; situated more or less halfway between tip of snout and eye (EN/NS 0.89–1.15 in males, 0.87–1.29 in females), separated from each other by distance subequal to or larger than distance between eye and nostril (NN/EN 0.96–1.15 in males, 0.96–1.11 in females); eye directed anterolaterally, moderately protruding, relatively small (ED/HL 0.24–0.30 in males, 0.25–0.29 in females), its diameter much shorter than snout (ED/SL 0.54–0.67 in males, 0.51–0.63 in females); interorbital distance subequal to upper eyelid width (IO/EW 0.80–1.09 in males, 0.81–1.10 in females) and smaller than internarial distance (IO/ NN 0.65–0.74 in males, 0.60–0.82 in females); tympanum and its annulus distinctly visible, separated from eye by about one-fourth to two-fifths of its diameter (ET/TD 0.27–0.39 in males, 0.29–0.39 in females); tympanum diameter smaller than eye diameter (TD/ED 0.68–0.81 in males, 0.70–0.84 in females); upper jaw with dentition; choanae small, rounded, located far anterolaterally at margins of roof of mouth; vomer teeth in

two short rows, separated from each other by distance about three times length of individual row; tongue long and narrow, bilobed for about one-fifth of its length, free distally for one-third its length; median lingual process absent; vocal sac in males paired, lateral; external vocal sac aperture as a longitudinal, posterolaterally orientated slit, inferior, terminating at level of ventral edge of insertion of arms; internal vocal sac apertures rounded, situated close to corner of mouth.

Dorsal surfaces of head, trunk and limbs finely shagreened; dorsum with four or five longitudinal dermal ridges on each side; median ridge extending from interorbital region almost to vent, postpalpebral and external ridges from posterior edge of upper eyelid to insertion of leg; laterodorsal ridge extending from level of insertion of arm to groin; sacral ridge extending from about one head length anterior to vent to vent, in some specimens absent; external ridge forming anterior part of supratympanic fold; posterior part of supratympanic fold less distinct, branching off from external dorsal ridge posterior to tympanum in wide angle and extending posterolaterally to insertion of arm; infratympanic fold thick and conspicuous, almost straight-lined, extending from ventral edge of eye to level of arm insertion, meeting with supratympanic fold; ventral side of limbs and body smooth except areolate postaxial side of thigh; distinct transverse fold between arms on ventral side; ventral side of trunk and head densely covered with more or less evenly scattered small, pointed tubercles in males.

Forelimbs moderately sturdy; hand relatively small (HND/SUL 0.22–0.26 in males, 0.22–0.24 in females); tips of fingers rounded, not enlarged into disks but slightly swollen volarly; transverse dorsal skin ridge separating ultimate from other phalanges on each finger; relative length of fingers: I ≤ II < IV < III; subarticular tubercles rounded, well developed, numbering one on Fingers I and II, two on Fingers III and IV, proximal tubercles on Fingers III and IV larger and more prominent than distal ones; finger webbing absent; thenar tubercle distinct, elongated, large, two-thirds length of metacarpal of Finger I; inner palmar tubercle on proximal third of metacarpal region of Fingers II and III, oval, flat; outer palmar tubercle on proximal half of metacarpal region of Finger IV, elongated, slightly more prominent than inner palmar tubercle; one supernumerary metacarpal tubercle between palmar or thenar tubercles and proximal subarticular tubercles on Fingers I, II, and IV, two on Finger III; callous longitudinal ridges between subarticular tubercles on Fingers III and IV and between subarticular tubercles and finger tips on all fingers; nuptial pads in males covering almost entire dorsal surfaces of Fingers I and II and preaxial half of dorsal side of Finger III.

Hindlimbs sturdy, very long (LEG/SUL 1.77–2.05 in males, 1.80–2.06 in females); knee reaching to insertion of forelimbs and tibio-tarsal articulation reaching slightly more than one snout length beyond tip of snout when legs adpressed forwardly to body; tibiofibula long (TFL/SUL 0.56–0.65 in males, 0.56–0.66 in females), longer than thigh (TFL/THL 1.08–1.18 in males, 1.05–1.26 in female); heels overlapping each other considerably when knees flexed and thighs held perpendicularly to median plane; two low longitudinal ridges on plantar side of tarsus between heel and metatarsal tubercles; foot slightly shorter than or equal in

length to tibiofibula (FOT/TFL 0.94–1.01 in males, 0.92–1.01 in females); relative length of toes: I < II < III < V < IV; toe tips rounded, not enlarged into disks but slightly swollen plantarly; transverse dorsal skin ridge separating ultimate from other phalanges on each toe; subarticular tubercles numbering one on Toes I and II, two on Toes III and V, and three on Toe IV; low callous ridges between subarticular tubercles, and between subarticular tubercles and toe tips; pedal webbing formula I(1.75–2)-2.25II1.5-3III1.75-(3–3.25)IV3-(1–1.5)V; inner metatarsal tubercle very prominent, shovel-like, large, more than half as long as metatarsus of Toe I; outer metatarsal tubercle faintly visible, rarely prominent and pointed.

Ptychadena uzungwensis

Body moderately sturdy, widest at temporal region, slightly tapering to groin; head large (HL/SUL 0.36–0.39 in males, 0.37 in females; HW/SUL 0.32–0.35 in males, 0.32 in female), longer than wide (HL/HW 1.06–1.23 in males, 1.16 in female); snout long (SL/HL 0.50–0.52 in males, 0.50 in female), pointed in dorsal view, rounded in profile, considerably projecting beyond lower jaw, much longer than wide (SL/EE 1.30–1.53 in males, 1.56 in female); canthus rostralis distinct between eye and nostril, straight-lined; loreal region oblique, strongly concave; nostrils rounded, directed dorsolaterally; situated more or less half-way between tip of snout and eye (EN/NS 0.91–1.03 in males, 0.97 in female), separated from each other by distance subequal to or shorter than distance between eye and nostril (NN/EN 0.82–0.94 in males, 0.89 in female); eyes directed anterolaterally, moderately protruding, relatively small (ED/HL 0.27–0.32 in males, 0.28 in female), its diameter much shorter than snout (ED/SL 0.55–0.63 in males, 0.56 in female); interorbital distance subequal to or larger than upper eyelid width (IO/EW 0.97–1.24 in males, 1.19 in female) and smaller or subequal to internarial distance (IO/NN 0.80–1.06 in males, 0.78 in female); tympanum and its annulus distinctly visible, separated from eye by about two-fifths to half of its diameter (ET/TD 0.36–0.47 in males, 0.41 in female); tympanum diameter smaller than eye diameter (TD/ED 0.70–0.83 in males, 0.77 in female); upper jaw with dentition; choanae small, rounded, located far anterolaterally at margins of roof of mouth; vomer teeth in two short rows, separated from each other by distance about two and half times length of individual row; tongue long and narrow, bilobed for about one-third of its length, free distally for one-third its length; median lingual process absent; vocal sac in males paired, lateral; external vocal sac aperture as a longitudinal, posterolaterally orientated slit, semi-inferior, terminating at level of centre of insertion of arms; internal vocal sac apertures rounded, situated close to corner of mouth.

Dorsal surfaces of head, trunk and limbs finely shagreened; dorsum with four longitudinal dermal ridges on each side; median ridge extending from dorsal side of snout between nostrils almost to vent; postpalpebral ridge extending from posterior portion of upper eyelid, external ridge from level of tympanum to insertion of leg; laterodorsal ridge extending from level of tympanum to groin; external ridge forming anterior part of supratympanic fold; posterior part of supratympanic fold less distinct, branching off from external dorsal

ridge posterior to tympanum in wide angle and extending posterolaterally to insertion of arm; infratympanic fold thick and conspicuous, almost straight-lined, extending from ventral edge of eye to level of arm insertion, meeting with supratympanic fold; ventral side of limbs and body smooth except areolate postaxial side of thigh; distinct transverse fold between arms on ventral side; ventral side of trunk and head densely covered with more or less evenly scattered small, pointed, very small tubercles in males.

Forelimbs moderately sturdy; hand relatively small (HND/SUL 0.22–0.24 in males, 0.21 in female); tips of fingers rounded, not enlarged into disks but slightly swollen volarly; transverse dorsal skin ridge separating ultimate from other phalanges on each finger; relative length of fingers: II = IV ≤ I < III; subarticular tubercles rounded, well developed, numbering one on Fingers I and II, two on Fingers III and IV, proximal tubercles on Fingers III and IV larger and more prominent than distal ones; finger webbing absent; thenar tubercle distinct, elongated, large, half as long as metacarpal of Finger I; inner palmar tubercle on proximal third of metacarpal of Finger III, small, roundish, flat; outer palmar tubercle on proximal third of metacarpal of Finger IV, elongated, more prominent than inner palmar tubercle; one supernumerary metacarpal on Fingers I and IV, two on Finger II, and two to four on Finger III between palmar or thenar tubercles and proximal subarticular tubercles; callous longitudinal ridges between subarticular tubercles on Fingers III and IV and between subarticular tubercles and finger tips on all fingers; nuptial pads in males covering almost entire dorsal surfaces of metacarpal and proximal phalanx of Fingers I and II and preaxial halves of dorsal sides of metacarpal and [in Rwandan specimens but not in paratype and specimens from Kivu and Burundi] proximal portion of proximal phalanx of Finger III.

Hindlimbs sturdy, very long (LEG/SUL 1.84–2.04 in males, 1.87 in female); knee reaching to insertion of forelimbs and tibio-tarsal articulation reaching slightly more than one snout length beyond tip of snout when legs adpressed forwardly to body; tibiofibula long (TFL/SUL 0.59–0.66 in males, 0.62 in female), longer than thigh (TFL/THL 1.12–1.20 in males, 1.16 in female); heels overlapping each other considerably when knees flexed and thighs held perpendicularly to median body plane; two low longitudinal ridges on plantar side of tarsus between heel and metatarsal tubercles, postaxial one less distinct than preaxial one; foot shorter than or subequal in length to tibiofibula (FOT/TFL 0.94–1.03 in males, 1.07 in female); relative length of toes: I < II < III < V < IV; toe tips rounded, not enlarged into disks but slightly swollen plantarly; transverse dorsal skin ridge separating ultimate from other phalanges on each toe; subarticular tubercles numbering one on Toes I and II, two on Toes III and V, and three on Toe IV; low callous ridges between subarticular tubercles, and between subarticular tubercles and toe tips; pedal webbing formula I2-(2.25–2.5)II1.5-3^{-}III(1.75–2^{-})-3IV3-(1^{+}–1.25)V; inner metatarsal tubercle prominent, elongated, large, half as long as metatarsus of Toe I; outer metatarsal tubercle faintly visible.

Appendix to Chapter V

Appendix 6. Comparative material examined.

Hyperolius acuticeps Ahl, 1931: "Konde-Nike, D. O. A." [Ukone Unyika, Tanzania] (ZMB 36093, lectotype; ZMB 65176, paralectotype).

Hyperolius acutirostris Buchholz & Peters in Peters, 1975: "Cameruns"/"Kamerun" (ZMB 8470, 65177, syntypes).

Hyperolius castaneus Ahl, 1931: "Rugegewald, 2000 m" [= Nyungwe Forest, Rwanda] (ZMB 36114, holotype of *Hyperolius adolphi-friederici* Ahl, 1931); Nyungwe Forest, Rwanda (ZMB 77537 [ex JMD 725]; JMD 600, 601, 602, 603, 604, 634, 640, 641, 676, 680–684, 728, 729, R1–R19, K1–K9); Gishwati Forest, Rwanda (JMD 581–583, 569, 586–590); Lac Ngezi, Volcano National Park, Rwanda (JMD Nge1).

Hyperolius chrysogaster Laurent, 1950: Bitale, 1750 m, west of Kahusi, Kalehe territory, Kivu, Democratic republic of the Congo (RMCA A0-074-B-1, holotype); Kamituga, 1050 m, Mwenga territory, Kivu, DRC (RMCA A0-074-B-22–27, paratypes).

Hyperolius cf. *cinnamomeoventris* Bocage, 1866: Butare, Rwanda (ZMB 77533 [ex JMD 651], 1S–8S); Mwogo River, Rwanda (JMD 635).

Hyperolius diaphanus Laurent, 1972: Itula, 650 m, Shabunda territory, Kivu, Democratic Republic of the Congo (RMCA A1-105-B-21, paratype).

Hyperolius discodactylus Ahl, 1931: "Rugegewald" [= Nyungwe Forest, Rwanda] (ZMB 36089, holotype; ZMB 77536 [ex JMD 724]; JMD 606, 632, 633); Gishwati Forest, Rwanda (JMD 567, 568); "Ruwenzori, 1800 m" (ZMB 39008, 74944, syntypes of *Hyperolius alticola* Ahl, 1931).

Hyperolius frontalis Laurent, 1950: Kamituga, 1050 m, Mwenga territory, Kivu, Democratic Republic of the Congo (RMCA A1-105-B-1–20, paratypes).

Hyperolius kivuensis Ahl, 1931: "Kivu-See" [= Lake Kivu] (ZMB 36098, holotype); "Usumbura, Tanganjika" (ZMB 36111, lectotype of *Hyperolius simus* Ahl, 1931); Butare, Rwanda (ZMB 77532 [ex JMD 560]; JMD 529, 531, 561); Gitarama, Rwanda (JMD 612); Ruhengeri, Rwanda (JMD 580), Rukarara River, Rwanda (JMD 628); Mwogo River, Rwanda (JMD 637, 709); Mugesera wetland, Rwanda (JMD 714, 715); Akagera wetland, Rwanda (JMD 720); Kahuzi-Biega National Park, Democratic Republic of the Congo (JMD 616).

Hyperolius lateralis Laurent, 1940: Butare, Rwanda (ZMB 77534 [ex JMD 654]); JMD 646, 650, 652, 653, 655, 660–664); Mugesera wetland, Rwanda (JMD 710); Rukarara River, Rwanda (JMD 665–669, lat1–lat3).

Hyperolius leucotaenius Laurent, 1950: Kiandjo (1850–1950 m), Mwenga territory, Kivu, Democratic Republic of the Congo (RMCA A0-074-B-28, holotype).

Hyperolius nitidulus Peters, 1875: "Chinchoxo, Afr. Escp." (ZMB 9173 [2 specs.]).

Hyperolius substriatus Ahl, 1931: "[Magrotto bei] Tanga", Tanzania (ZMB 39007 [4 specs.], possible syntypes/paralectotypes).

Hyperolius viridiflavus-complex: "Mohasi-See, Ruanda" [= Lake Muhazi, Rwanda] (ZMB 36105, 75606, syntypes of *Hyperolius irregularis* Ahl, 1931; JMD 564, 565); "Kissenji" (ZMB 26089, holotype of *Hyperolius koehli* Ahl, 1931); "Urwald auf der Insel Kwidjwi (Kivu-See)" [= Ile Idjwi/Kwidjwi Island, Lake Kivu, DRC] (ZMB 52449, holotype of *Hyperolius kwidjwiensis* Ahl, 1931); "Kiwu-See" [=Lake Kivu] (ZMB 39100, holotype of *Hyperolius macrodactylus* Ahl, 1931); "Niansa (Ruanda), 1500 m" (ZMB 39010, holotype of *Hyperolius monticola* Ahl, 1931); "D.C.A.E. Karissimbi, Bambuswald und Waldwiesen, ca. 2400 m" (ZMB 39002, 39005, 74953–74956, syntypes of *Hyperolius multicolor* Ahl, 1931), "Zentrales D.O.A." [= central German East Africa] (ZMB 39000 [4 specs.], syntypes of *Hyperolius phrynoderma* Ahl, 1931); "Vulkangebiet nordöstlich des Kivusees" (ZMB 36117, paratype of *Hyperolius punctatissimus* Ahl, 1931); "Usumbura, Tanganjika"(ZMB 65179, 65180, paralectotypes of *Hyperolius simus* Ahl, 1931); "Insel Kwidjwi, Kivu-See" (ZMB 46521, possible syntype or paratype of *Hyperolius variabilis* Ahl, 1931); "Bukoba, N-Tansania" (ZMB 43587–43590, possible paratypes of *Hyperolius punctatissimus* Ahl, 1931; ZMB 71201); Butare, Rwanda (JMD 530, 532, 533); Gitarama, Rwanda (ZMB 77535 [ex JMD 613]); Ruhengeri, Rwanda (JMD 579); Mizingo, Rwanda (ZMB 77531 [ex JMD I8]; JMD 642–645, 647, 648, I1–I7, I9–I19); Rugesi Marais, Rwanda (JMD 691); Mukungwa River, Rwanda (JMD 623); Rukarara River, Rwanda (JMD 629, 630); Mwogo River, Rwanda (JMD 636, 721); Lac Ngezi, Volcano National Park, Rwanda (JMD 638); Mugesera wetland, Rwanda (JMD 713); Akagera wetland, Rwanda (JMD 720); Kahuzi-Biega National Park, Democratic Republic of the Congo (JMD 615, 617).

Hyperolius sp. (*nasutus*-group): Butare, Rwanda (ZMB 77141, 77142, 77423–77429); Mugesera wetland, Rwanda (ZMB 77143, 77144); Akagera wetland, Rwanda (ZMB 77145).

Appendices to Chapter VI

Appendix 7. Gazetteer of localities.

Locality	Latitude	Longitude
Akagera wetland, Rwanda	2°13'27.7" N	30°49'39.1" E
Bicuar National Park, Angola	15°06'03.4" S	14°50'21.0" E
Bumba, DRC	2°10'57.0" N	22°28'08.4" E
Butare, Rwanda	2°37'08.0" N	29°45'06.9" E
Bwindi Impenetrable Forest, Uganda	1°03'00.0" S	29°43'00.0" E
Calandula (Duque de Bragança), Angola	9°04'45.0" S	15°47'45.0" E
Camp Nimba, Guinea	7°41'54.9" N	8°23'55.1" W
Chelinda Camp, Malawi	10°34'59.9 S	33°48'00.0 E
Chongoni Forest Reserve, Malawi	14°19'59.9 S	34°15'00.0" E
Congo River near Yekela, site 1, DRC	0°47'25.7" N	24°17'52.5 E
Congo River near Yekela, site 2, DRC	0°49'42.6" N	24°16'26.5" E
Congo River near Yekela, site 3, DRC	0°49'18.7" N	24°16'40.1" E
Congo River near Nganda Kona, site 4, DRC	2°02'13.7" N	22°47'09.6" E
Congo River near Ngengele, site 5, DRC	2°03'32.2" N	22°42'08.4" E
Congo River near Bomani, site 6, DRC	1°16'35.8" N	23°45'38.3 E
Congo River near Bomani, site 7, DRC	1°17'28.9" N	23°45'41.0"E
Congo River near Bomani, site 8, DRC	1°15'18.5" N	23°44"03.4" E
Congo River near Lulu, site 9, DRC	1°15'11.4" N	23°39'49.3" E
Congo River near Lieki, site 10, DRC	0°39'33.3" N	24°11'08.3" E
Congo River near Lieki, site 11, DRC	0.°41'29.8" N	24°11'58.8" E
Congo River near Lieki, site 12, DRC	0.°41'05.1" N	24°13'29.0" E
Elephant's Camp, Mozambique	15°39'19.0" S	30°41'09.0" E
Fungurume, DRC	10°37'00.0" S	26°20'00.0" E
Gatiko, Central African Republic	5°04'43.0" N	20°40'02.0" E
Himo Road, Arusha, Tanzania	3°21'29.6" S	36°50'15.3" E
Humpata, Angola	14°14'17.3" S	13°25'59.9" E
Ikelenge Nature Reserve, Zambia	11°14'17.3" S	24°16'05.3" E
Impongui, Republic of Congo	1°04'36.6" N	17°17'58.8" E
Kakamega, Kenya	0°16'59.9" N	34°45'00.0" E
Kalumbila River bridge, Zambia	12°14'13.2" S	25°20'44.5" E
Kalumbila River, Zambia	12°16'45.1" S	25°19'06.7" E
Kangandala, Angola	9°49'30.4" S	16°54'44.1" E
Kaningina Forest Reserve, Malawi	11°28'00.0 S	34°06'00.0" E
Kaparotta, Botswana	19°00'24.0" S	22°55'36.6" E
Karonga, Malawi	11°22'00.0" S	34°10'00.0" E

Appendix 7. (continued)

Locality	Latitude	Longitude
Kérouané, Guinea	9°14'24.7" N	8°59'52.0" W
Kisanfu River, DRC	10°48'28.0" S	25°59'24.0" E
Kisangani, DRC	0°30'36.7" N	25°12'19.4" E
Konsankoro, Guinea	9°01'59.9" N	9°00'00.0" W
Korombadou/Tourou, Guinea	9°16'24.6" N	9°08'54.5" W
Lake Carumbo, Angola	7°43'59.9" S	19°55'59.9" E
Lamto, Ivory Coast	0°13'03.4"N	5°01'29.6" W
Loma Mountains Forest Reserve, Sierra Leone (LOM100)	9°12'45.6" N	11°08'40.1" W
Loma Mountains Forest Reserve, Sierra Leone (LOM99)	9°12'58.8" N	11°07'52.8" W
Lupiro, Tanzania	8°25'29.3" S	36°41'33.1" E
Madehani, Tanzania	9°21'00.0" S	34°01'59.9" E
Mare d'hivenage, Guinea	7°39'38.1" N	8°22'49.2" W
Mkambati Nature Reserve, South Africa	31°16'39.9" S	30°00'27.3" E
Monkey Bay, Malawi	14°04'37.0" S	34°55'34.2"E
Mont Béro Forest Reserve, Guinea	8°08'41.0" N	8°33'11.1" W
Mugesera wetland, Rwanda	2°12'34.1" N	30°15'26.4" E
National Forestry School, Libreville, Gabon	0°03'37.1" N	9°20'14.2" E
Himo Road, Arusha, Tanzania	3°21'29.6" S	36.83758 E
Mkambati Nature Reserve, South Africa	31°16'39.9" S	30°50'15.3" E
Nimba Mountains, Guinea (MTN255-256)	7°40'22.8" N	8°21'55.0" W
Nimba Mountains, Guinea (MTN93)	7°41'47.7" N	8°23'55.1" W
Nimini Forest Reserve, Sierra Leone	8°29'58.7" N	11°05'30.1 W
Nyanga Flats, low bridge, Zimbabwe	18°10'00.6" S	32°42'48.6" E
Plain of Vera, Gabon	2°49'43.0" S	10°12'57.2" E
Port Edward, South Africa	31°04'00.8" S	30°11'16.1" E
Quantum Mine Camp, Zambia	12°14'13.2" S	25°20'44.5" E
Rhodes Dam, Nyanga NP, Zimbabwe	18°17'20.3" S	32°43'24.4" E
Rundu, Namibia	17°55'12.0" S	19°45'00.0" E
Savanne de But, Guinea	7°41'17.1" N	8°25'44.5" W
Satellite Camp, Mozambique	15°39'11.0" S	30°35'14.0" E
Setecama Road, Gabon	2°43'58.9" S	9°58'41.8" E
Uemba Road, Gabon	2°44'49.4" S	10°00'17.9" E
Vintukutu Forest Reserve, Malawi	10°45'00.0" S	34°16'00.0" E
Vumbura, Botswana	18°58'38.0" S	22°53'41.3" E
Xigera, Botswana	19°23'03.8" S	22°43'44.6" E
Zootecnica Plateau, Angola	14°57'56.9" S	13°20'40.5" E

Appendix 8. Comparative measurements (in mm) of representative specimens. For abbreviations see Materials and Methods.

Specimen	SUL	TFL	LEG	FOT	HND	HW	HL	ED	EN	NS	NN	SL	EE	IO	EW	THL
Hyperolius acuticeps MCZ A-137085, female	22.0	10.7	31.8	8.8	5.9	6.6	7.3	1.9	2.0	1.4	1.8	3.1	4.3	3.0	3.0	10.5
Hyperolius acuticeps MCZ A-137086, female	21.4	11.4	32.9	9.9	5.8	7.0	6.4	2.2	1.6	0.9	2.2	3.0	4.5	2.6	1.4	10.9
Hyperolius adspersus USNM 578142, male	19.5	10.5		8.9	5.4	6.4	5.3	2.0	1.6	0.7	1.9	2.7	4.2	1.9	1.3	9.7
Hyperolius benguellensis ZMB 77272, male	19.3	10.2	29.9	8.5	5.7	5.7	6.4	2.1	1.8	1.1	1.9	2.7	4.0	4.0	2.4	11.2
Hyperolius benguellensis male	19.3	10.1	31.9	7.4	5.2	5.8	5.2	1.7	1.9	1.1	2.1	2.9	3.7	2.3	1.4	10.1
Hyperolius benguellensis ZMB 77275, female	23.7	12.0	34.3	9.7	6.2	7.4	6.3	2.1	1.9	1.3	2.2	3.3	4.5	3.1	1.2	11.0
Hyperolius benguellensis ZMB 77271, female	23.5	13.4	37.2	10.3	6.7	7.3	7.1	2.2	2.3	1.1	2.1	3.4	5.2	2.8	1.8	13.3
Hyperolius friedemanni ZMB 76095, paratype, female	21.0	11.7		14.9	6.0	5.1	7.1	1.8	2.3	1.2	1.7	2.8	5.7	2.7	2.5	11.0
Hyperolius howelli SAIAB 118979, holotype, male	20.2	11.3	27.5	7.9	4.9	6.2	6.4	2.3	1.6	0.8	1.7	3.1	4.1	2.7	2.8	8.9
Hyperolious howelli SAIAB 118980-1, paratype, female	19.0	11.0	29.7	8.7	4.0	5.5	6.7	2.1	1.6	0.8	1.7	2.6	3.8	2.7	2.8	8.1
Hyperolius howelli SAIAB 118980-2, paratype, male	21.0	10.3	32.6	8.5	5.6	6.1	7.1	2.2	1.5	0.9	1.9	2.0	3.8	2.8	1.0	9.6
Hyperolius igbettensis ZMB 77410, female	21.1	12.2	33.5	10.5	5.9	5.5	7.0	2.0	2.1	1.1	1.9	3.0	4.1	2.1	3.0	10.8
Hyperolius inyangae ZMB 77276, holotype male	21.7	10.4	31.0	8.8	5.2	6.4	8.1	1.9	2.0	2.0	2.3	3.7	4.5	2.4	2.1	10.7
Hyperolius inyangae ZMB 77277, paratype, female	21.6	11.1	31.9	7.0	5.3	7.0	7.4	2.2	1.9	1.3	2.1	3.4	4.1	2.5	1.3	11.1
Hyperolius inyangae ZMB 77278, paratype, male	19.7	10.3	27.7	8.8	5.1	6.5	6.8	2	1.8	1.3	1.9	2.9	3.9	2.4	1.7	10.2

.....*continued on the next page*

Appendix 8. (continued)

Specimen	SUL	TFL	LEG	FOT	HND	HW	HL	ED	EN	NS	NN	SL	EE	IO	EW	THL
Hyperolius inyangae ZMB 77279, paratype, male	19.0	9.6	26.4	7.3	4.9	6.0	6.6	1.7	1.8	1.4	2.1	3.3	4.0	2.7	1.6	9.5
Hyperolius jacobseni ZMB 77280, holotype, male	21.3	11.1	34.4	8.7	6.0	5.9	7.2	1.8	1.6	1.0	2.1	3.1	4.2	2.9	1.0	10.6
Hyperolius jacobseni ZMB 77281, paratype, female	19.5	11.2	23.3	8.4	6.7	6.2	6.7	1.9	1.9	0.8	1.9	3.0	4.1	2.5	1.0	10.5
Hyperolius lamottei ZMB 76526, male	21.8	9.0	25.7	7.3	4.0	7.1	7.1	2.7	1.6	1.0	1.9	3.1	3.2	2.9	3.1	9.4
Hyperolus lupiroensis ZMB 77299, holotype, female	23.0	12.0	37.7	8.7	5.9	5.4	7.4	2.6	1.8	1.0	1.7	3.6	3.3	2.4	0.8	11.6
Hyperolius nasicus ZMB 77310, male	18.9	9.1	26.6	7.6	5.2	5.1	6.2	1.7	1.7	1.0	1.7	2.8	3.9	2.4	2.3	8.0
Hyperolius nasutus SAIAB 186001, male	16.6	7.9	24.7	5.5	4.1	5.2	4.6	2.1	1.2	1.0	1.9	1.4	2.6	4.4	2.3	6.9
Hyperolius poweri PEM A 9545, male	20.4	10.6	30.6	8.6	5.5	6.2	6.1	2.1	1.9	1.3	2.2	3.0	4.0	2.4	2.1	10.1
Hyperolius poweri PEM A 9546, male	19.1	10.8	33.5	9.1	5.4	6.9	6.2	2.1	1.7	0.8	1.8	2.5	4.2	2.7	1.0	9.6
Hyperolius rwandae ZMB 7721, holotype, male	18.9	10.2	30.7	8.3	5.5	5.8	6.3	2	1.7	1.2	1.9	2.8	3.6	2.2	1.3	
Hyperolius rwandae ZMB77222, paratype, male	18.8	10	30.2	8.1	5.1	5.6	6.3	2	1.7	1.1	1.7	2.4	3.2	2.2	1.2	
Hyperolius rwandae ZMB 77224, paratype, male	19.2	9.7	30	8.1	4.8	5.9	6.1	1.8	1.7	1.1	1.8	2.8	3.4	2.3	1.1	
Hyperolius rwandae ZMB 77225, paratype, male	20.2	10.4	31.8	8.5	5.2	5.7	6.2	1.9	1.9	1	1.9	2.8	3.7	2.2	1.3	
Hyperolius rwandae ZMB 77423, paratype, male	19.3	10.1	29.8	8.5	5.4	5.7	6.5	2.3	1.7	1.3	1.9	2.8	3.7	2.5	1.1	
Hyperolius rwandae ZMB 77424, paratype, male	20.2	10.4	32	8.5	6.2	6	6.8	2.3	1.8	1.3	2	3.1	4	2.6	1.4	
Hyperolius rwandae ZMB 77426, paratype, male	19.4	10.6	32.5	8.2	5.2	5.9	6.5	2.2	1.9	1.2	2	3	3.9	2.3	1.4	

……*continued on the next page*

Appendix 8. (continued)

Specimen	SUL	TFL	LEG	FOT	HND	HW	HL	ED	EN	NS	NN	SL	EE	IO	EW	THL
Hyperolius rwandae ZMB 77427, paratype, male	19.9	10.5	33	9.1	5.9	5.8	6.9	2.3	1.9	1.2	2.1	2.9	4.1	2.5	1.3	
Hyperolius rwandae ZMB 77428, paratype, male	19.1	10.1	29.8	8.9	5.7	5.6	6.3	2.2	1.6	1.3	1.9	2.8	3.5	2.5	1.2	
Hyperolius rwandae ZMB 77429, paratype, male	18.6	10.3	31.9	8.7	5.4	5.3	6.1	2.4	1.8	1.2	1.8	2.7	3.9	2.4	1.3	
Hyperolius rwandae ZMB 77685, paratype, male	18.4	8.7	27.8	6.8	4.7	5	5.8	2	1.7	1.1	1.8	2.7	3.4	2	1	
Hyperolius rwandae ZMB 77686, paratype, male	20	9.9	30.7	8.5	5.8	5.9	6.5	2.3	1.7	1.2	1.8	3	3.6	2.2	1.4	
Hyperolius rwandae ZMB 77687, paratype, male	19.6	10	31.7	8.7	5.6	5.9	6.6	2.2	1.9	1.4	1.9	3.1	3.9	2.5	1.2	
Hyperolius rwandae ZMB 77688, paratype, male	21.3	11.3	33.9	8.9	5.7	6.2	6.6	2.5	2	1.4	2.1	3.1	4.1	2.6	1.2	
Hyperolius rwandae ZMB 77689, paratype, male	20.1	10.2	31.6	8.6	5.4	6	6.5	2.4	1.9	1.3	2.2	3.1	4.2	2.3	1.5	
Hyperolius rwandae ZMB 77746, paratype, male	22	11.7	34.4	9	6.1	6.9	6.5	2.3	2	1.4	2	3.1	4.1	2.5	1.6	
Hyperolius rwandae ZMB 77747, paratype, male	20	10.2	31.1	8.5	5.7	6.1	6.5	2.1	1.7	1.2	1.9	2.9	3.9	2.3	1.3	
Hyperolius rwandae ZMB 77748, paratype, male	20.6	10.5	31.8	8.4	5.5	6.3	6.6	3.3	1.9	1.3	2.1	2.9	4.1	2.5	1.3	
Hyperolius rwandae ZMB 77223, paratype, female	19	8.8	27.5	7.1	4.2	5.3	6.2	1.8	1.7	1	1.8	2.6	3.5	2.2	1.2	
Hyperolius rwandae ZMB 77425, paratype, female	18.2	10	29.7	8.2	4.8	5.2	5.6	2.2	1.5	1.1	1.8	2.8	3.2	2.1	1	
Hyperolius rwandae ZMB 77684, paratype, female	20.4	9.9	29.5	8.2	5.4	5.7	6.4	2.3	2	1.2	2	3	4	2.4	1.4	
Hyperolius viridis ZMB 76096, male	20.9	11.2	30.4	8.9	4.0	7.1	6.4	2.7	1.9	1.6	1.8	2.7	2.8	2.4	3.1	10.3

Appendices to Chapter VIII

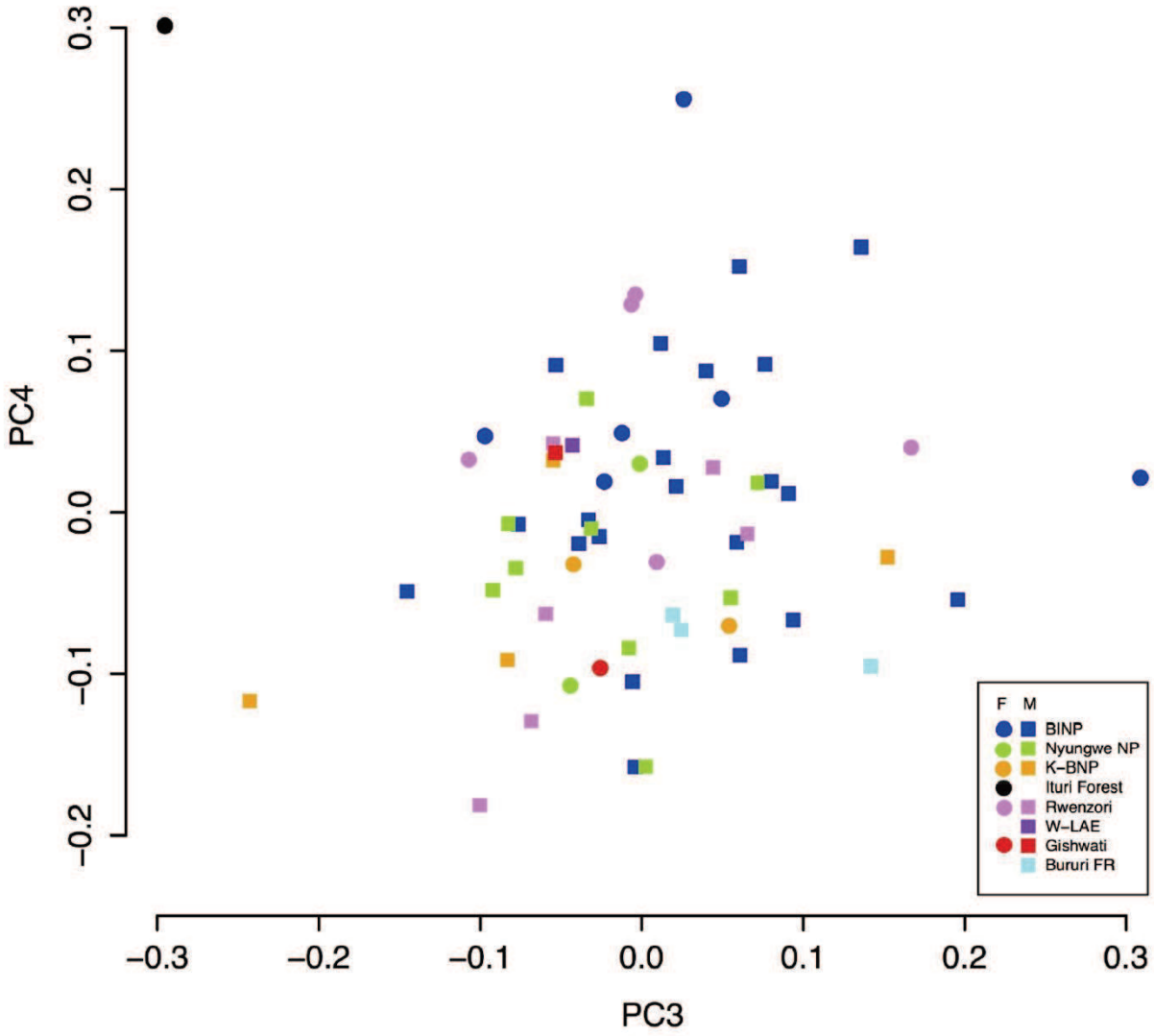

FIGURE S1. Biplot of Principal Components three and four of a PCA on all fourteen log transformed morphometric variables for *H. discodactylus.*

TABLE S1. External morphological measurements (in mm) of snout-vent length (SVL), snout-urostyle length (SUL), internarial space (INS), eyelid width (EW), canthus length (CL), interorbital space (IOS), head width (HW), 1st to 5th toe length (1-5TL), femur length (FL), tibia length (TibL) and tarsus length (TarL). Webbing formula and collection locality is also given where BINP refers to Bwindi Impenetrable National Park, W-LAE to west of Lake Albert-Edward and K-BNP to Kahuzi-Biega National Park.

Voucher	Locality	Sex	SVL	SUL	INS	EW	CL	IOS	HW	1TL	2TL	3TL	4TL	5TL	FL	TibL	TarL	Toe webbing formula
CAS 180449	BINP	M	24.9	24.0	1.9	1.8	2.4	3.8	7.8	4.1	5.0	8.4	11.2	9.4	11.9	12.9	6.9	1 (1), 2i (1 1/4), 2e (0), 3i(1), 3e(1/4), 4i(1 3/4), 4e(1 3/4), 5(0)
CAS 180450	BINP	M	28.2	28.1	2.6	1.8	2.8	3.6	10.6	4.5	6.9	10.4	12.6	10.6	13.7	13.7	8.2	1 (1/2), 2i (1), 2e (1/4), 3i(1 1/4), 3e(1/4), 4i(1 3/4), 4e(1 1/2), 5(0)
CAS 180451	BINP	M	26.9	26.7	2.4	2.1	2.6	3.6	10.4	4.8	6.5	10.1	12.5	10.4	13.9	14.0	8.2	1 (1), 2i (1 1/2), 2e (1/4), 3i(1 3/4), 3e(3/4), 4i(1 1/2), 4e(1 1/4), 5(0)
CAS 180452	BINP	M	28.5	27.6	2.5	2.2	2.7	3.9	10.7	4.6	6.8	10.3	12.9	11.0	13.7	14.0	8.1	1 (3/4), 2i (1 1/4), 2e (1/4), 3i(1 1/2), 3e(1/2), 4i(1 3/4), 4e(1 1/4), 5(1/4)
CAS 180453	BINP	M	27.8	27.3	2.8	2.2	2.6	4.0	10.6	5.0	6.6	11.0	13.1	10.9	15.0	14.6	7.7	1 (1/2), 2i (1), 2e (1/4), 3i(1 1/4), 3e(3/4), 4i(1 1/4), 4e(1 1/4), 5(1/4)
CAS 180454	BINP	M	27.1	26.9	2.3	2.0	2.6	3.7	9.5	4.6	7.4	10.7	13.0	11.4	13.4	13.3	7.6	1 (1), 2i (1 1/4), 2e (1/4), 3i(1), 3e(3/4), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 180455	BINP	M	29.2	29.0	2.7	2.1	2.4	3.7	9.7	4.9	7.5	10.9	12.2	11.9	14.0	14.6	7.9	1 (3/4), 2i (1 1/4), 2e (1/4), 3i(1 1/4), 3e(3/4), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 180456	BINP	M	30.7	30.0	2.6	2.4	2.8	4.4	10.2	5.4	7.3	10.6	13.7	12.1	15.0	14.9	8.2	1 (1/4), 2i (1 1/4), 2e (0), 3i(1 1/2), 3e(0), 4i(1 3/4), 4e(1 1/4), 5(0)
CAS 180457	BINP	M	28.3	27.8	2.9	2.4	2.9	4.4	10.4	5.6	8.0	11.9	14.4	12.3	15.0	15.7	8.5	1 (1/4), 2i (1), 2e (1 1/4), 3i(1), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 180458	BINP	M	30.3	29.8	2.5	2.0	2.9	4.0	10.4	5.0	7.8	11.6	15.2	12.6	15.1	15.6	8.2	1 (1/2), 2i (1), 2e (1/4), 3i(1 3/4), 3e(1/4), 4i(1 1/2), 4e(1 1/2), 5(0)
CAS 180459	BINP	F	31.7	31.2	3.1	2.3	3.3	4.2	11.9	6.0	10.0	12.7	16.3	13.5	16.6	17.3	10.6	1 (1/2), 2i (1), 2e (1/4), 3i(1 1/4), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 180460	BINP	F	31.1	31.1	2.6	2.3	2.9	4.4	11.3	5.3	7.9	11.4	15.4	12.1	15.2	16.1	8.7	1 (3/4-1), 2i (1 3/4), 2e (1/4), 3i(1), 3e(1 1/2), 4i(3/4), 4e(1 1/2), 5(1/2)
CAS 180461	BINP	F	32.2	31.9	3.5	2.7	2.7	3.9	12.0	5.4	8.0	12.6	15.5	12.9	15.2	16.2	8.9	1 (1/2), 2i (1), 2e (1/2), 3i(1), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(1/4)
CAS 180462	BINP	M	28.1	28.1	2.8	2.0	2.5	3.8	10.3	5.1	7.5	11.9	14.7	12.1	14.2	14.8	8.1	1 (1), 2i (1 1/4), 2e (1/2), 3i(1 1/2), 3e(3/4), 4i(1 1/2), 4e(1 1/2), 5(1/4)
CAS 180463	BINP	M	30.9	29.2	2.5	2.6	2.7	3.8	10.8	5.7	7.9	11.2	14.0	11.0	14.3	15.4	9.3	1 (1/4), 2i (1 1/4), 2e (0), 3i(1), 3e(1/4), 4i(1 3/4), 4e(1 3/4), 5(0)
CAS 180464	BINP	M	28.6	28.3	2.4	2.3	2.7	4.4	10.0	5.4	7.2	10.4	12.8	10.0	14.2	13.1	7.6	1 (3/4), 2i (1), 2e (0), 3i(1), 3e(3/4), 4i(1 3/4), 4e(1 3/4), 5(0)
CAS 180485	BINP	M	28.9	28.7	2.8	2.0	2.6	4.2	10.3	5.4	7.7	11.4	14.1	11.5	14.7	14.4	8.0	1 (1/4), 2i (1), 2e (1/4), 3i(1 1/4), 3e(1/2), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 180486	BINP	M	28.4	28.2	2.5	2.3	2.9	3.5	10.0	4.6	7.2	11.2	13.8	11.3	14.8	14.9	8.3	1 (3/4), 2i (1 1/4), 2e (1/4), 3i(1), 3e(1/2), 4i(1 1/2), 4e(1 1/2), 5(1/4)
CAS 180488	BINP	M	29.8	28.7	2.8	2.6	3.1	4.2	10.5	4.6	6.8	10.0	12.9	10.7	13.0	14.1	7.5	1 (1), 2i (1 1/4), 2e (3/4), 3i(1 3/4), 3e(3/4-1), 4i(2), 4e(1 3/4), 5(0)
CAS 196153	Ituri	F	31.5	31.8	2.4	1.9	3.6	4.2	10.5	5.5	6.3	10.5	12.8	10.5	15.6	16.3	10.2	1 (1 1/4), 2i (1 1/4), 2e (3/4), 3i(1 1/2), 3e(1/2), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 202046	BINP	M	30.3	29.8	2.2	2.2	3.0	4.3	11.0	6.0	8.7	12.3	15.1	13.2	14.7	14.9	8.4	1 (1/4), 2i (1 1/4), 2e (1/4), 3i(1 1/4), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 202068	BINP	M	28.6	29.5	2.7	2.5	3.0	4.2	10.2	5.3	8.0	11.5	14.2	12.3	13.7	14.1	7.8	1 (1/4), 2i (1), 2e (0), 3i(1 1/4), 3e(3/4), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 202069	BINP	F	30.2	30.0	2.6	2.7	3.3	4.4	11.1	4.7	7.5	11.6	14.8	11.7	16.0	16.1	10.2	1 (3/4), 2i (1 1/4), 2e (1/4), 3i(1), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 202070	BINP	M	28.1	27.7	2.8	2.3	2.3	3.7	10.4	4.9	7.1	10.6	14.3	11.8	14.1	14.4	7.9	1 (1/2), 2i (1 1/4), 2e (1/4), 3i(1 1/4), 3e(3/4), 4i(1 1/4), 4e(1 1/4), 5(1/2)
CAS 202071	BINP	F	34.9	34.1	3.5	2.5	3.6	5.0	12.7	5.8	8.6	12.8	16.4	13.2	16.9	17.1	9.2	1 (1/4), 2i (1), 2e (0), 3i(1), 3e(1/4), 4i(1), 4e(1), 5(0)
CAS 202072	BINP	M	29.8	29.9	2.6	2.1	2.8	4.1	10.0	4.5	6.9	10.4	14.3	12.2	14.0	14.1	7.8	1 (3/4), 2i (1), 2e (1/4), 3i(1 1/4), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 202256	BINP	M	29.2	28.1	2.5	1.9	2.3	3.9	10.5	4.9	7.6	11.1	14.0	11.7	14.5	15.1	7.6	1 (1/4), 2i (1), 2e (0), 3i(1), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 202257	BINP	M	28.2	27.6	2.5	1.9	2.7	3.5	9.9	5.1	7.7	10.2	13.7	11.6	14.3	14.5	7.6	1 (3/4), 2i (1), 2e (1/4), 3i(1 1/4), 3e(3/4), 4i(1 1/4), 4e(1 1/4), 5(0)
CAS 202258	BINP	F	31.1	30.7	2.7	2.2	3.5	4.8	11.7	5.4	7.7	11.4	15.0	12.2	14.7	15.1	7.6	1 (3/4), 2i (1), 2e (1/2), 3i(1 3/4), 3e(3/4), 4i(1 3/4), 4e(1 3/4), 5(1/4)
MCZ A-17634	W-LAE	m	32.2	32.1	2.8	2.2	2.7	4.9	10.2	5.0	6.9	11.4	14.5	12.0	15.7	16.5	10.4	1 (1), 2i (1 1/4), 2e (1/4), 3i(1 1/2), 3e(1/4), 4i(1 1/2), 4e(1 1/2), 5(0)

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TABLE S1. (Continued)

Voucher	Locality	Sex	SVL	SUL	INS	EW	CL	IOS	HW	1TL	2TL	3TL	4TL	5TL	FL	TibL	TarL	Toe webbing formula
MCZ A-25172	Rwenzori	F	35.9	35.0	3.3	2.7	3.8	5.5	12.4	5.8	9.0	12.6	16.6	12.7	16.7	16.5	10.2	1 (1), 2i (1 1/4), 2e (0), 3i(1 1/4), 3e(0), 4i(1 1/4), 4e(1 1/4), 5(0)
MCZ A-39371	Rwenzori	M	30.3	28.9	2.8	2.4	2.7	4.9	9.7	4.7	6.8	10.6	13.6	11.3	13.9	15.3	7.9	1 (1), 2i (1 1/4), 2e (1 1/4), 3i(1 1/4), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
MCZ A-39372	Rwenzori	m	30.0	28.5	2.7	2.0	2.6	5.0	9.6	4.8	7.4	10.4	13.3	10.8	14.4	14.5	7.6	1 (1), 2i (1), 2e (1/4), 3i(1), 3e(0), 4i(1 1/4), 4e(1 1/4), 5(0)
MCZ A-39373	Rwenzori	m	29.7	28.6	3.0	2.2	2.7	4.8	10.0	4.6	6.6	10.2	12.6	10.3	14.4	14.7	8.3	1 (1), 2i (1 1/4), 2e (1 1/4), 3i(1 1/4), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
MCZ A-39374	Rwenzori	M	28.8	27.7	2.9	1.9	2.8	4.5	9.7	5.1	7.5	10.4	13.2	11.1	13.0	14.1	8.1	1 (1), 2i (1 1/4), 2e (1/2), 3i(1), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
MCZ A-39375	Rwenzori	M	30.2	28.7	2.9	2.1	3.1	4.9	9.8	4.6	7.3	10.5	13.5	10.6	13.6	14.6	8.2	1 (1), 2i (1 1/4), 2e (1), 3i(1 1/4), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
MCZ A-39376	Rwenzori	M	29.0	28.6	2.5	1.9	2.5	4.6	9.6	5.1	7.7	11.0	13.2	11.0	13.4	14.5	7.4	1 (1), 2i (1), 2e (1/4), 3i(1 1/4), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
MTSN6832	K-BNP	M	30.6	31.4	2.3	2.0	2.8	5.4	10.4	5.2	8.4	11.0	14.3	11.7	14.9	16.2	8.7	1 (1/2), 2i (1 1/4), 2e (1/4), 3i(1 1/4), 3e(1/2), 4i(1 1/2), 4e(1 1/2), 5(1/2)
MTSN6835	K-BNP	M	33.4	31.6	2.8	2.3	2.9	5.2	10.5	6.0	8.5	11.3	15.6	12.1	16.3	16.7	8.9	1 (3/4), 2i (1 1/4), 2e (0), 3i(1 1/4), 3e(1 1/2), 4i(1 3/4), 4e(1 3/4), 5(0)
MTSN6849	K-BNP	M	30.9	30.6	3.1	2.7	2.8	4.8	11.2	5.4	8.2	11.3	15.2	12.7	15.2	16.6	8.7	1 (3/4), 2i (1 1/4), 2e (1/4), 3i(1 1/2), 3e(3/4), 4i(1 3/4), 4e(1 3/4), 5(0)
MTSN6850	K-BNP	F	36.7	36.5	3.1	2.6	3.4	5.7	12.4	6.2	9.2	12.6	16.1	14.2	17.8	19.4	9.3	1 (3/4), 2i (1 1/4), 2e (1/4), 3i(1 1/4), 3e(3/4), 4i(1 3/4), 4e(1 1/2), 5(0)
MTSN6856	K-BNP	F	34.6	33.6	3.4	2.6	3.1	5.3	12.8	6.6	9.2	12.5	16.0	13.3	17.2	18.8	9.4	1 (3/4), 2i (1 1/4), 2e (0), 3i(1 1/4), 3e(1/2), 4i(1 1/2), 4e(1 1/4), 5(0)
MTSN6867	K-BNP	M	33.4	34.6	2.8	2.5	3.1	5.2	10.8	5.6	8.1	12.0	15.0	11.8	16.8	17.2	9.4	1 (3/4), 2i (1 1/4), 2e (1/4), 3i(1 1/4), 3e(1/4), 4i(1 3/4), 4e(1 1/2), 5(1/4)
MTSN7364	Nyungwe	M	30.7	30.3	2.8	2.3	3.3	5.1	11.1	5.8	7.9	11.9	15.2	12.8	15.0	16.6	8.4	1 (1), 2i (1 1/4), 2e (1/2), 3i(1 1/2), 3e(1), 4i(1 3/4), 4e(1 1/2), 5(1/4)
MTSN7365	Nyungwe	F	34.7	34.0	3.2	2.5	3.2	5.4	13.0	7.1	10.2	13.4	15.6	12.5	17.5	18.7	9.4	1 (1/4), 2i (1), 2e (0), 3i(1 1/2), 3e(1/4), 4i(1 1/4), 4e(1 1/4), 5(0)
MTSN7366	Nyungwe	M	31.7	30.9	2.6	2.3	3.0	5.0	11.3	6.0	8.0	11.9	15.2	12.1	15.3	15.5	8.5	1 (1/2), 2i (1 1/4), 2e (1/2), 3i(1 1/2), 3e(3/4), 4i(1 1/2), 4e(1 1/4), 5(0)
MTSN7367	Nyungwe	F	34.3	32.7	3.4	2.3	3.4	5.0	12.1	6.0	8.0	12.5	16.6	12.7	15.8	16.8	9.2	1 (1), 2i (1 1/4), 2e (3/4), 3i(1 1/2), 3e(1), 4i(1 3/4), 4e(1 1/2), 5(0)
MTSN7369	Nyungwe	M	30.4	30.6	3.1	2.2	2.7	5.0	11.1	6.0	8.2	12.0	15.1	12.9	16.6	17.1	8.6	1 (1), 2i (1 1/4), 2e (1/2), 3i(1 1/2), 3e(3/4), 4i(1 3/4), 4e(1 1/2), 5(0)
SL440	Rwenzori	F	37.4	37.2	3.5	3.0	3.2	5.3	12.6	6.0	8.9	12.3	16.4	14.5	16.8	17.5	10.2	1 (1/4), 2i (3/4), 2e (0), 3i(3/4), 3e(0), 4i(1), 4e(3/4), 5(0)
SL453	Rwenzori	F	35.8	35.6	3.5	2.5	3.2	5.6	11.8	7.0	8.1	11.8	16.9	13.1	16.0	16.2	9.9	1 (1/4), 2i (1), 2e (0), 3i(1 1/4), 3e(1/4), 4i(1), 4e(3/4), 5(0)
ZMB36089	Nyungwe	M	33.9	33.9	3.1	2.6	3.0	5.6	12.1	5.0	7.4	10.8	12.9	11.6	15.9	15.8	8.1	1 (1/4), 2i (1), 2e (1/4), 3i(1 1/4), 3e(3/4), 4i(1 1/4), 4e(1 1/4), 5(0)
ZMB39008	Rwenzori	F	36.5	34.9	3.3	2.7	3.9	5.1	12.7	6.3	9.0	12.8	17.4	13.5	17.2	18.4	10.0	1 (1/4), 2i (3/4), 2e (0), 3i(1), 3e(1/2), 4i(11/4), 4e(1 1/4), 5(0)
ZMB74944	Rwenzori	F	30.0	30.5	2.9	2.3	3.4	5.4	11.6	5.7	8.1	11.6	15.1	12.2	15.5	16.4	9.1	1 (1), 2i (3/4), 2e (0), 3i(1), 3e(1/2), 4i(11/4), 4e(1 1/4), 5(0)
ZMB77536	Nyungwe	M	29.0	28.2	2.6	2.0	2.6	4.9	11.1	4.5	7.3	10.7	13.1	10.7	13.9	15.2	8.8	1 (1), 2i (1 1/4), 2e (0), 3i(1 1/2), 3e(3/4), 4i(1 1/2), 4e(1 1/2), 5(1/4)
ZMB78947	Gishwati	F	38.4	38.8	3.3	2.7	3.5	6.4	13.8	6.3	10.1	15.4	18.8	15.6	18.3	19.4	10.0	1 (1), 2i (1 1/4), 2e (0), 3i(1 1/4), 3e(0), 4i(1 1/4), 4e(1 1/4), 5(0)
ZMB78948	Gishwati	M	33.6	32.6	2.7	2.4	2.9	5.0	11.3	5.3	7.8	11.7	15.6	11.7	16.3	18.0	9.8	1 (1), 2i (1 1/4), 2e (0), 3i(1 1/2), 3e(1/2), 4i(1 1/2), 4e(1 1/2), 5(0)
ZMB78949	Nyungwe	M	34.1	34.1	3.1	2.4	3.0	5.3	11.8	5.9	8.9	12.5	16.5	12.4	16.2	16.6	9.1	1 (1), 2i (1 1/4), 2e (1/4), 3i(1 1/2), 3e(1/2), 4i(1 3/4), 4e(1 1/2), 5(1/4)
ZMB78950	Nyungwe	M	30.4	29.4	2.8	2.1	3.0	4.3	11.1	4.8	8.0	10.9	14.2	10.9	14.4	14.6	9.0	1 (1), 2i (1 1/4), 2e (0), 3i(1 1/4), 3e(0), 4i(1 1/2), 4e(1 1/2), 5(0)
ZMB78951	Nyungwe	M	31.9	30.3	2.6	2.2	2.9	4.3	10.4	5.1	8.0	10.9	14.0	10.8	13.8	15.2	7.7	1 (3/4), 2i (1), 2e (1/4), 3i(1 1/4), 3e(1), 4i(1 1/2), 4e(1 1/2), 5(0)
ZMB78952	Bururi	M	30.0	29.2	2.8	2.4	2.5	4.8	10.2	4.2	7.4	11.4	13.2	11.3	13.5	14.1	7.3	1 (1), 2i (1 1/4), 2e (1/2), 3i(1 1/4), 3e(1/2), 4i(1 1/2), 4e(1 1/2), 5(1/4)
ZMB78953	Bururi	M	30.7	29.0	2.8	2.2	2.7	4.8	11.2	5.1	7.3	11.4	13.2	11.5	13.6	14.8	8.0	1 (1), 2i (1), 2e (0), 3i(1 1/4), 3e(0), 4i(1 1/4), 4e(1 1/4), 5(0)
ZMB78955	Bururi	M	30.1	28.8	2.9	2.1	2.6	4.6	10.2	4.9	7.2	10.6	13.7	10.7	14.5	14.7	7.8	1 (1), 2i (1 1/4), 2e (0), 3i(1 1/2), 3e(1/4), 4i(1 1/2), 4e(1 1/2), 5(0)
ZMB78956	Nyungwe	M	33.5	30.9	3.0	2.6	2.8	5.4	11.4	5.4	8.5	12.0	14.9	12.2	15.8	16.5	9.5	1 (1), 2i (1 1/4), 2e (0), 3i(1 1/2), 3e(1/4), 4i(1 1/2), 4e(1 1/4), 5(0)

TABLE S2. PCA scores on log transformed morphometric data of *H. discodactylus* with a summary statistics of the importance of components.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
SUL	-0.272	0.036	-0.124	0.012	-0.130	0.094	-0.090	0.187	-0.298	0.213	-0.740	-0.023	-0.305	-0.258
INS	-0.249	-0.499	0.162	-0.256	0.628	0.375	-0.029	-0.172	0.027	0.165	-0.013	-0.040	-0.043	0.008
EW	-0.207	-0.650	0.362	0.037	-0.526	-0.298	0.121	-0.111	-0.049	0.008	0.051	-0.003	-0.061	-0.019
CL	-0.252	-0.098	-0.149	0.805	0.341	-0.311	-0.129	-0.011	-0.020	0.067	0.094	-0.104	-0.051	-0.007
IOS	-0.348	-0.207	-0.807	-0.290	-0.103	-0.138	-0.038	-0.091	0.028	-0.188	0.102	-0.002	0.075	0.093
HW	-0.237	-0.104	0.137	0.038	0.067	0.011	-0.011	0.616	0.412	-0.325	-0.237	0.210	0.389	0.050
1TL	-0.315	0.247	0.093	-0.064	0.197	-0.179	0.828	-0.072	-0.184	-0.162	-0.024	-0.028	0.023	-0.065
2TL	-0.294	0.300	0.136	-0.202	-0.009	-0.359	-0.122	-0.278	0.560	0.438	-0.146	-0.086	0.040	0.090
3TL	-0.237	0.154	0.163	-0.127	0.033	-0.021	-0.205	0.119	0.190	-0.453	0.256	-0.126	-0.679	-0.193
4TL	-0.281	0.180	0.144	-0.023	0.026	-0.048	-0.264	-0.222	-0.294	-0.052	0.111	0.799	0.075	-0.030
5TL	-0.246	0.165	0.243	-0.145	0.015	-0.095	-0.364	-0.027	-0.444	-0.225	-0.014	-0.511	0.382	0.184
FL	-0.243	0.091	0.013	0.039	-0.137	0.214	0.097	0.348	-0.119	0.317	0.214	0.036	-0.254	0.714
TibL	-0.273	0.094	-0.029	-0.009	-0.164	0.227	0.011	0.275	-0.032	0.389	0.468	-0.108	0.231	-0.570
TarL	-0.255	0.116	0.004	0.340	-0.305	0.613	0.064	-0.438	0.236	-0.233	-0.104	-0.082	0.103	0.057
Standard deviation	0.316	0.109	0.097	0.092	0.072	0.070	0.058	0.045	0.044	0.038	0.033	0.030	0.027	0.022
Proportion of Variance	0.658	0.079	0.063	0.056	0.034	0.032	0.022	0.013	0.013	0.009	0.007	0.006	0.005	0.003
Cumulative Proportion	0.658	0.736	0.799	0.855	0.889	0.921	0.943	0.957	0.970	0.979	0.986	0.992	0.997	1.000

TABLE S3. Posterior probabilities of LDA classifications.

	locality	sex	a priori group	alticola_f	alticola_m	discodactylus_f	discodactylus_m	miss-matches
CAS 180450	BINP	M	alticola_m	0.01	0.93	0.00	0.06	
CAS 180451	BINP	M	alticola_m	0.00	1.00	0.00	0.00	
CAS 180452	BINP	M	alticola_m	0.00	0.93	0.00	0.07	
CAS 180453	BINP	M	alticola_m	0.00	0.95	0.00	0.05	
CAS 180454	BINP	M	alticola_m	0.00	1.00	0.00	0.00	
CAS 180455	BINP	M	alticola_m	0.00	1.00	0.00	0.00	
CAS 180456	BINP	M	alticola_m	0.00	1.00	0.00	0.00	
CAS 180457	BINP	M	alticola_m	0.00	0.98	0.00	0.02	
CAS 180458	BINP	M	alticola_m	0.00	0.99	0.00	0.01	
CAS 180459	BINP	F	alticola_f	1.00	0.00	0.00	0.00	
CAS 180460	BINP	F	alticola_f	0.21	0.30	0.00	0.49	*
CAS 180461	BINP	F	alticola_f	0.92	0.00	0.02	0.07	
CAS 180462	BINP	M	alticola_m	0.00	0.93	0.00	0.07	
CAS 180463	BINP	M	alticola_m	0.00	0.99	0.00	0.01	
CAS 180464	BINP	M	alticola_m	0.00	1.00	0.00	0.00	
CAS 180485	BINP	M	alticola_m	0.00	0.95	0.00	0.05	
CAS 180486	BINP	M	alticola_m	0.00	1.00	0.00	0.00	
CAS 180488	BINP	M	alticola_m	0.00	0.95	0.00	0.05	
CAS 202046	BINP	M	alticola_m	0.01	0.90	0.00	0.09	
CAS 202068	BINP	M	alticola_m	0.00	0.99	0.00	0.01	
CAS 202069	BINP	F	alticola_f	0.58	0.34	0.00	0.09	
CAS 202070	BINP	M	alticola_m	0.00	0.99	0.00	0.01	
CAS 202071	BINP	F	alticola_f	0.95	0.00	0.05	0.00	
CAS 202072	BINP	M	alticola_m	0.00	1.00	0.00	0.00	
CAS 202256	BINP	M	alticola_m	0.00	0.81	0.00	0.19	
CAS 202257	BINP	M	alticola_m	0.00	1.00	0.00	0.00	
CAS 202258	BINP	F	alticola_f	0.73	0.01	0.00	0.27	
MCZ A-25172	Rwenzori	F	alticola_f	1.00	0.00	0.00	0.00	
MCZ A-39371	Rwenzori	M	alticola_m	0.00	0.99	0.00	0.01	
MCZ A-39372	Rwenzori	M	alticola_m	0.00	0.98	0.00	0.02	

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TABLE S3. (Continued)

	locality	sex	a priori group	alticola_f	alticola_m	discodactylus_f	discodactylus_m	miss-matches
MCZ A-39373	Rwenzori	M	alticola_m	0.00	0.97	0.00	0.03	
MCZ A-39374	Rwenzori	M	alticola_m	0.00	0.99	0.00	0.01	
MCZ A-39375	Rwenzori	M	alticola_m	0.00	0.91	0.00	0.09	
MCZ A-39376	Rwenzori	M	alticola_m	0.00	0.97	0.00	0.03	
MTSN6832	K-BNP	M	alticola_m	0.00	0.68	0.00	0.32	
MTSN6835	K-BNP	M	alticola_m	0.00	0.98	0.00	0.02	
MTSN6849	K-BNP	M	alticola_m	0.15	0.38	0.00	0.47	*
MTSN6850	K-BNP	F	alticola_f	0.99	0.00	0.00	0.01	
MTSN6856	K-BNP	F	alticola_f	0.75	0.00	0.23	0.02	
MTSN6867	K-BNP	M	alticola_m	0.02	0.89	0.00	0.09	
MTSN7364	Nyungwe	M	discodactylus_m	0.32	0.31	0.00	0.37	
MTSN7365	Nyungwe	F	discodactylus_f	0.00	0.00	1.00	0.00	
MTSN7366	Nyungwe	M	discodactylus_m	0.04	0.17	0.00	0.79	
MTSN7367	Nyungwe	F	discodactylus_f	0.69	0.00	0.31	0.00	*
MTSN7369	Nyungwe	M	discodactylus_m	0.03	0.61	0.00	0.36	*
SL440	Rwenzori	F	alticola_f	1.00	0.00	0.00	0.00	
SL453	Rwenzori	F	alticola_f	0.99	0.00	0.00	0.01	
ZMB36089	Nyungwe	M	discodactylus_m	0.10	0.00	0.00	0.90	
ZMB39008	Rwenzori	F	alticola_f	0.99	0.00	0.01	0.00	
ZMB74944	Rwenzori	F	alticola_f	0.71	0.01	0.00	0.28	
ZMB77536	Nyungwe	M	discodactylus_m	0.00	0.00	0.00	1.00	
ZMB78949	Nyungwe	M	discodactylus_m	0.39	0.00	0.02	0.59	
ZMB78950	Nyungwe	M	discodactylus_m	0.20	0.07	0.00	0.74	
ZMB78951	Nyungwe	M	discodactylus_m	0.00	0.83	0.00	0.17	*
ZMB78952	Bururi	M	discodactylus_m	0.00	0.03	0.00	0.97	
ZMB78953	Bururi	M	discodactylus_m	0.00	0.01	0.00	0.99	
ZMB78955	Bururi	M	discodactylus_m	0.00	0.82	0.00	0.18	*
ZMB78956	Nyungwe	M	discodactylus_m	0.03	0.01	0.00	0.96	
***a posteriori* placements**								
MCZ A-17634	W-LAE	M	discodactylus_m	0.00	0.99	0.00	0.00	*
ZMB78947	Gishwati	F	alticola_f	0.66	0.00	0.34	0.00	
ZMB78948	Gishwati	M	discodactylus_m	0.20	0.15	0.00	0.66	

TABLE S4. Contingency table of prior versus predicted classifications.

	alticola_f	alticola_m	discodactylus_f	discodactylus_m
alticola_f	12	0	0	1
alticola_m	0	30	0	1
discodactylus_f	1	0	1	0
discodactylus_m	0	3	0	9

TABLE S5. MANCOVA table testing investigator effect. Level of significances correspond to ** < 0.001 and * <0.05.

	Df	Pillai	approx F	num Df	den Df	P-value	level of significance
SUL	1	0.933	39.428	13	37	0.000	**
sex	1	0.582	3.970	13	37	0.001	**
morphotype	1	0.510	2.956	13	37	0.005	**
investigator	1	0.392	1.831	13	37	0.075	
SUL:sex	1	0.228	0.842	13	37	0.616	
SUL:morphotype	1	0.288	1.153	13	37	0.350	
sex:morphotype	1	0.300	1.218	13	37	0.306	
SUL:investigator	1	0.360	1.599	13	37	0.130	
sex:investigator	1	0.118	0.381	13	37	0.968	
SUL:sex:morphotype	1	0.276	1.083	13	37	0.403	
SUL:sex:investigator	1	0.236	0.877	13	37	0.582	
Residuals	49						

TABLE S6. ANCOVA tables for each morphometric variable. Level of significances correspond to ** < 0.001 and * <0.05.

	Df	Sum Sq	Mean Sq	F value	p-value	level of significance
SUL						
sex	1	0.200	0.200	51.077	0.000	**
morphotype	1	0.019	0.019	4.737	0.034	*
sex:morphotype	1	0.013	0.013	3.251	0.077	
Residuals	57	0.224	0.004			
Internarial						
SUL	1	0.358	0.358	58.630	0.000	**
sex	1	0.040	0.040	6.569	0.013	*
morphotype	1	0.002	0.002	0.323	0.572	
SUL:sex	1	0.014	0.014	2.243	0.140	
SUL:morphotype	1	0.000	0.000	0.073	0.788	
sex:morphotype	1	0.032	0.032	5.195	0.027	*
SUL:sex:morphotype	1	0.004	0.004	0.736	0.395	
Residuals	53	0.323	0.006			
eyelid						
SUL	1	0.354	0.354	48.452	0.000	**
sex	1	0.009	0.009	1.205	0.277	
morphotype	1	0.008	0.008	1.054	0.309	
SUL:sex	1	0.000	0.000	0.002	0.966	

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TABLE S6. (Continued)

	Df	Sum Sq	Mean Sq	F value	p-value	level of significance
SUL:morphotype	1	0.000	0.000	0.007	0.932	
sex:morphotype	1	0.012	0.012	1.705	0.197	
SUL:sex:morphotype	1	0.000	0.000	0.000	0.990	
Residuals	53	0.387	0.007			
canthus						
SUL	1	0.399	0.399	69.914	0.000	**
sex	1	0.086	0.086	15.133	0.000	**
morphotype	1	0.000	0.000	0.000	0.985	
SUL:sex	1	0.007	0.007	1.159	0.286	
SUL:morphotype	1	0.001	0.001	0.211	0.648	
sex:morphotype	1	0.013	0.013	2.348	0.131	
SUL:sex:morphotype	1	0.002	0.002	0.429	0.515	
Residuals	53	0.332	0.006			
head width						
SUL	1	0.316	0.316	246.081	0.000	**
sex	1	0.044	0.044	34.348	0.000	**
morphotype	1	0.029	0.029	22.610	0.000	**
SUL:sex	1	0.004	0.004	2.876	0.096	
SUL:morphotype	1	0.000	0.000	0.384	0.538	
sex:morphotype	1	0.009	0.009	6.904	0.011	*
SUL:sex:morphotype	1	0.002	0.002	1.583	0.214	
Residuals	53	0.068	0.001			
1st toe						
SUL	1	0.419	0.419	64.482	0.000	**
sex	1	0.019	0.019	2.952	0.092	
morphotype	1	0.000	0.000	0.063	0.803	
SUL:sex	1	0.001	0.001	0.111	0.740	
SUL:morphotype	1	0.016	0.016	2.401	0.127	
sex:morphotype	1	0.003	0.003	0.473	0.495	
SUL:sex:morphotype	1	0.017	0.017	2.631	0.111	
Residuals	53	0.344	0.006			
2nd toe						
SUL	1	0.346	0.346	73.262	0.000	**
sex	1	0.015	0.015	3.167	0.081	
morphotype	1	0.005	0.005	1.124	0.294	
SUL:sex	1	0.001	0.001	0.268	0.607	
SUL:morphotype	1	0.001	0.001	0.312	0.579	
sex:morphotype	1	0.002	0.002	0.410	0.525	
SUL:sex:morphotype	1	0.019	0.019	3.971	0.051	
Residuals	53	0.251	0.005			
3rd toe						
SUL	1	0.236	0.236	91.071	0.000	**
sex	1	0.014	0.014	5.463	0.023	*
morphotype	1	0.003	0.003	1.012	0.319	
SUL:sex	1	0.002	0.002	0.785	0.380	
SUL:morphotype	1	0.000	0.000	0.016	0.900	
sex:morphotype	1	0.000	0.000	0.046	0.830	
SUL:sex:morphotype	1	0.006	0.006	2.492	0.120	
Residuals	53	0.138	0.003			

TABLE S6. (Continued)

	Df	Sum Sq	Mean Sq	F value	p-value	level of significance
4th toe						
SUL	1	0.356	0.356	123.402	0.000	**
sex	1	0.023	0.023	7.985	0.007	*
morphotype	1	0.000	0.000	0.002	0.962	
SUL:sex	1	0.000	0.000	0.122	0.729	
SUL:morphotype	1	0.001	0.001	0.400	0.530	
sex:morphotype	1	0.001	0.001	0.197	0.659	
SUL:sex:morphotype	1	0.001	0.001	0.218	0.643	
Residuals	53	0.153	0.003			
5th toe						
SUL	1	0.271	0.271	84.600	0.000	**
sex	1	0.015	0.015	4.680	0.035	*
morphotype	1	0.002	0.002	0.607	0.439	
SUL:sex	1	0.004	0.004	1.365	0.248	
SUL:morphotype	1	0.002	0.002	0.550	0.462	
sex:morphotype	1	0.001	0.001	0.443	0.508	
SUL:sex:morphotype	1	0.000	0.000	0.138	0.711	
Residuals	53	0.170	0.003			
Femur						
SUL	1	0.308	0.308	161.564	0.000	**
sex	1	0.005	0.005	2.551	0.116	
morphotype	1	0.000	0.000	0.002	0.965	
SUL:sex	1	0.002	0.002	1.046	0.311	
SUL:morphotype	1	0.004	0.004	1.944	0.169	
sex:morphotype	1	0.000	0.000	0.000	0.999	
SUL:sex:morphotype	1	0.002	0.002	1.085	0.302	
Residuals	53	0.101	0.002			
tibia						
SUL	1	0.372	0.372	151.504	0.000	**
sex	1	0.005	0.005	1.874	0.177	
morphotype	1	0.004	0.004	1.613	0.210	
SUL:sex	1	0.002	0.002	0.643	0.426	
SUL:morphotype	1	0.000	0.000	0.004	0.951	
sex:morphotype	1	0.001	0.001	0.274	0.603	
SUL:sex:morphotype	1	0.005	0.005	2.080	0.155	
Residuals	53	0.130	0.002			
tarsal length						
SUL	1	0.311	0.311	67.504	0.000	**
sex	1	0.018	0.018	3.814	0.056	
morphotype	1	0.001	0.001	0.282	0.598	
SUL:sex	1	0.006	0.006	1.258	0.267	
SUL:morphotype	1	0.006	0.006	1.298	0.260	
sex:morphotype	1	0.001	0.001	0.213	0.646	
SUL:sex:morphotype	1	0.003	0.003	0.599	0.442	
Residuals	53	0.244	0.005			

Table S7. Localities and coordinates used to map the distribution of *H. discodactylus*.

Institution code	Catalogue number	Country	Locality	State/Province	Geo-referenced?	Latitude	Longitude
CAS	180449	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180450	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180451	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180452	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180453	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180454	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180455	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180456	Uganda	BINP: Munyaga River, ca 3.5 km S of	Rukungiri Dist.		-1.00975	29.620695
CAS	180476	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180477	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180478	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180479	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180480	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180481	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180482	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180483	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180484	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180485	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180486	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180487	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180488	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	196153	DRC	Zone of Mambasa (Ituri Forest), Epulu	Haut-Zaire Prov.	yes	-1.8722222	29.472778
CAS	202046	Uganda	BINP, Munyaga River, ca 100 m downstream of Munyaga Falls	Rukungiri Dist.		-1.0051944	29.61575
CAS	202047	Uganda	BINP, Munyaga River, ca 100 m downstream boundary)	Rukungiri Dist.		-1.0051944	29.61575
CAS	180471	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180472	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180473	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180474	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180475	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695

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Table S7. (continued)

Institution code	Catalogue number	Country	Locality	State/Province	Geo-referenced?	Latitude	Longitude
CAS	180476	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180477	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180478	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180479	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180480	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180481	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180482	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180483	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180484	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180485	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180486	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180487	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	180488	Uganda	BINP: Munyaga River, ca 3.5 km S of Buhoma (ca 2 km S of forest reserve boundary)	Rukungiri Dist.		-1.00975	29.620695
CAS	196153	DRC	Zone of Mambasa (Ituri Forest), Epulu	Haut-Zaire Prov.	yes	-1.8722222	29.472778
CAS	202046	Uganda	BINP, Munyaga River, ca 100 m downstream of Munyaga Falls	Rukungiri Dist.		-1.0051944	29.61575
CAS	202047	Uganda	BINP, Munyaga River, ca 100 m downstream of Munyaga Falls	Rukungiri Dist.		-1.0051944	29.61575
CAS	202068	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202069	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202070	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202071	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202072	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202073	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202074	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202075	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202076	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202077	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202078	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
CAS	202253	Uganda	BINP, Kasiru North, upper E Fork Ntengere River	Rukungiri Dist.		-1.0185277	29.654055
CAS	202254	Uganda	BINP, Kasiru North, upper E Fork Ntengere River	Rukungiri Dist.		-1.0185277	29.654055
CAS	202255	Uganda	BINP, Kasiru North, upper E Fork Ntengere River	Rukungiri Dist.		-1.0185277	29.654055
CAS	202256	Uganda	BINP, Kasiru North, upper E Fork Ntengere River	Rukungiri Dist.		-1.0185277	29.654055
CAS	202257	Uganda	BINP, Kasiru North, upper E Fork Ntengere River	Rukungiri Dist.		-1.0185277	29.654055
CAS	202258	Uganda	BINP, Kasiru North, upper E Fork Ntengere River	Rukungiri Dist.		-1.0185277	29.654055
CAS	202259	Uganda	BINP, Kasiru North, upper E Fork Ntengere River	Rukungiri Dist.		-1.0185277	29.654055
CAS	204714	Uganda	BINP, Kasiru North, upper E Fork Ntengere River	Rukungiri Dist.		-1.0185277	29.654055
CAS	204724	Uganda	BINP, base of Munyaga Falls	Rukungiri Dist.		-1.0051944	29.61575
CAS	204726	Uganda	BINP, Munyaga River	Rukungiri Dist.		-1.00975	29.620695
FLMNH	113156	Rwanda		Kibuye Prefecture	yes	-1.8722222	29.472778
FLMNH	113157	Rwanda		Kibuye Prefecture	yes	-1.8722222	29.472778
FMNH	170502	Rwanda		Kibuye Territory	yes	-1.8722222	29.472778
FMNH	170503	Rwanda		Kibuye Territory	yes	-1.8722222	29.472778
IRSNBs	290328	DRC				0.35	29.816668
IRSNBs	351346	DRC				-1.3333334	29.033333
IRSNBs	371996	DRC				0.35	29.783333

Table S7. (continued)

Institution code	Catalogue number	Country	Locality	State/Province	Geo-referenced?	Latitude	Longitude
IRSNBs	396386	DRC				0.05	29.15
IRSNBs	455759	DRC				0.38333333	29.9
IRSNBs	514197	DRC				0.35	29.816668
IRSNBs	580626	DRC				-1.0833334	29.316668
KU	155086	Rwanda	Lutsiro	Kibuye	yes	-1.8722222	29.472778
KU	155087	Rwanda	Lutsiro	Kibuye	yes	-1.8722222	29.472778
KU	155088	Rwanda	Lutsiro	Kibuye	yes	-1.8722222	29.472778
KU	155089	Rwanda	Lutsiro	Kibuye	yes	-1.8722222	29.472778
LACM	50093	Rwanda	Lutsiro	Kibuye Territory	yes	-1.8722222	29.472778
LACM	50094	Rwanda	Lutsiro	Kibuye Territory	yes	-1.8722222	29.472778
LACM	50095	Rwanda	Lutsiro	Kibuye Territory	yes	-1.8722222	29.472778
LACM	50096	Rwanda	Lutsiro	Kibuye Territory	yes	-1.8722222	29.472778
LACM	50097	Rwanda	Lutsiro	Kibuye Territory	yes	-1.8722222	29.472778
LACM	50098	Rwanda	Lutsiro	Kibuye Territory	yes	-1.8722222	29.472778
LACM	50099	Rwanda	Lutsiro	Kibuye Territory	yes	-1.8722222	29.472778
LACM	50100	Rwanda	Lutsiro	Kibuye Territory	yes	-1.8722222	29.472778
LACM	50101	Rwanda	Lutsiro	Kibuye Territory	yes	-1.8722222	29.472778
LACM	50102	Rwanda	Lutsiro	Kibuye Territory	yes	-1.8722222	29.472778
MCZ	A-25172	Uganda	Mubuku [Mobuku] Valley, Ruwenzori Mtns, Uganda [VERBATIM ELEVATION:6800ft]	Kasese, Busongora		0.28837	30.115404
MCZ	A-39363	DRC	B Congo: Kiondo ya Kwanza nr Kalonge PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.332764	29.799628
MCZ	A-39364	DRC	B Congo: Kiondo ya Kwanza nr Kalonge PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.332764	29.799628
MCZ	A-39365	DRC	B Congo: Kiondo ya Kwanza nr Kalonge PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.332764	29.799628
MCZ	A-39366	DRC	B Congo: Kiondo ya Kwanza nr Kalonge PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.332764	29.799628
MCZ	A-39367	DRC	B Congo: Kiondo ya Kwanza nr Kalonge PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.332764	29.799628
MCZ	A-39368	DRC	B Congo: Kiondo ya Kwanza nr Kalonge PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.332764	29.799628
MCZ	A-39369	DRC	B Congo: Nyamwamba nr Kalonge PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39370	DRC	B Congo: Nyamwamba nr Kalonge PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39371	DRC	B Congo: Kalivina R PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.30741	29.785674
MCZ	A-39372	DRC	B Congo: Kalivina R PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.30741	29.785674
MCZ	A-39373	DRC	B Congo: Kalivina R PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.30741	29.785674
MCZ	A-39374	DRC	B Congo: Kalivina R PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.30741	29.785674
MCZ	A-39375	DRC	B Congo: Kalivina R PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.30741	29.785674
MCZ	A-39376	DRC	B Congo: Kalivina R PNA Kivu	Kivu	yes (Based on Laurent 1972)	0.30741	29.785674
MCZ	A-39970	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39971	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39972	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39973	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39974	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39975	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39976	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39977	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39978	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39979	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39980	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39981	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39982	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39983	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39984	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39985	DRC	Congo: Nyamwamba: nr Kalonge, Kivu [VERBATIM ELEVATION:2010m]	Kivu	yes (Based on Laurent 1972)	0.34586	29.794147
MCZ	A-39986	DRC	Congo: Kamusonde: nr Kalonge, PNA, Kivu [VERBATIM ELEVATION:1900m]	Kivu	yes (Based on Laurent 1972)	0.336782	29.798264
MCZ	A-39987	DRC	Congo: Kamusonge: nr Kalonge, PNA, Kivu [VERBATIM ELEVATION:1900m]	Kivu	yes (Based on Laurent 1972)	0.336782	29.798264

Table S7. (continued)

Institution code	Catalogue number	Country	Locality	State/Province	Geo-referenced?	Latitude	Longitude
MCZ	A-39988	DRC	Congo: Kamusonde: nr Kalonge, PNA, Kivu [VERBATIM ELEVATION:1900m]	Kivu	yes (Based on Laurent 1972: NOTE = Kamusonge!)	0.336782	29.798264
MCZ	A-39989	DRC	Congo: Kisuku: PNA: Kivu	Kivu	yes (Based on Laurent 1972: NOTE = Kisuhu!)	-0.085585	29.479477
MCZ	A-39990	DRC	Congo: Kisuku: PNA: Kivu	Kivu	yes (Based on Laurent 1972: NOTE = Kisuhu!)	-0.085585	29.479477
MCZ	A-39991	DRC	Congo: Kisuku: PNA: Kivu	Kivu	yes (Based on Laurent 1972: NOTE = Kisuhu!)	-0.085585	29.479477
MNHN	1968.153	Rwanda	Lutsiro, 2600 m	Kibuye	yes	-1.8722222	29.472778
MTSN	7209	Rwanda	Nyungwe National Park			-2.56753	29.23079
MTSN	7210	Rwanda	Nyungwe National Park			-2.739167	29.390361
MTSN	7211	Rwanda	Nyungwe National Park			-2.739167	29.390361
MTSN	7350	Rwanda	Nyungwe National Park			-2.476692335	29.15833787
MTSN	7364	Rwanda	Nyungwe National Park			-2.455324433	29.24949971
MTSN	7365	Rwanda	Nyungwe National Park			-2.455324433	29.24949971
MTSN	7366	Rwanda	Nyungwe National Park			-2.455324433	29.24949971
MTSN	7367	Rwanda	Nyungwe National Park			-2.455324433	29.24949971
MTSN	7369	Rwanda	Nyungwe National Park			-2.455324433	29.24949971
MTSN	6832	DRC	Kahuzi-Biega National Park			-2.327865651	28.728924
MTSN	6835	DRC	Kahuzi-Biega National Park			-2.327865651	28.728924
MTSN	6849	DRC	Kahuzi-Biega National Park			-2.327865651	28.728924
MTSN	6850	DRC	Kahuzi-Biega National Park			-2.327865651	28.728924
MTSN	6856	DRC	Kahuzi-Biega National Park			-2.327865651	28.728924
MVZ	220749	Uganda	BINP, Buhoma Rd., ca. 2.0 km S Forest Reserve boundary	Rukungiri District		-1	29.666666
MVZ	220753	Uganda	BINP, Buhoma Rd., ca. 2.0 km S Forest Reserve boundary	Rukungiri District		-1	29.666666
MVZ	220754	Uganda	BINP, Buhoma Rd., ca. 2.0 km S Forest Reserve boundary	Rukungiri District		-1	29.666666
MVZ	220761	Uganda	BINP, Munyaga River, ca. 3.5 km S Buhoma Rd., ca. 2.0 km S Forest Reserve boundary	Rukungiri District		-1	29.666666
MVZ	220762	Uganda	BINP, Munyaga River, ca. 3.5 km S Buhoma Rd., ca. 2.0 km S Forest Reserve boundary	Rukungiri District		-1	29.666666
Ptiman	NA	Uganda	Kayonsa Forest, S-W. Kigazi		yes (Based on Laurent 1950)	-1.07	30.07
RMCA	?	Rwanda	Kibga, au sud du Visoke [alt. 2400m], II.1935		yes	-1.48635	29.5443
RMCA	?	Rwanda	Kibga, au sud du Visoke [alt. 2400m], II.1935		yes	-1.48635	29.5443
RMCA	?	Rwanda	Kibga, au sud du Visoke [alt. 2400m], II.1935		yes	-1.48635	29.5443
RMCA	74018B2918-19	DRC	Riv. Kandiko, afflt. dr. de la riv. Butahu, près de Kalonge, alt.2020m.		yes (Based on Laurent 1972)	0.330966	29.787022
RMCA	74018B2920-22	DRC	Riv. Katauleko, afflt. dr. de la riv. Butahu, près de Kalonge, alt.2060m.		yes (Based on Laurent 1972)	0.333517	29.787426
RMCA	74018B2923-42	DRC	Riv. Katauleko, afflt. dr. de la riv. Butahu, près de Kalonge, alt.2060m.		yes (Based on Laurent 1972)	0.333517	29.787426
RMCA	74018B2951-54	DRC	Riv. Lusilube, afflt. de la riv. Semliki, rég. de Mwenda, piste de Mwenda-Katuka, alt.1360m.		yes (Based on Laurent 1972)	0.415523	29.772401
RMCA	B.58388-89	DRC	Burunga, près des lacs Mokoto, alt.2190m., P.N.A.		yes (based on Laurent 1950)	-1.30806	29.012842
RMCA	B.58390	DRC	Burunga, près des lacs Mokoto, alt.2190m., P.N.A.		yes (based on Laurent 1950)	-1.30806	29.012842
RMCA	B.58391	Rwanda	Kundhuru-Ya-Tshuwe, alt.2600m., P.N.A.		yes (Laurent 1950)	-1.405356	29.633804
RMCA	B.60188	DRC	Lwiro, alt.2000m.			-2.26159	28.7801
S. Lötters	SL453	Uganda	Rwenzori Mts.			0.422283	30.009041
ZMB	39008	DRC	Rwenzori, 1800m		yes (Based on Schubotz 1912)	0.424544	29.79893
ZMB	74944	DRC	Rwenzori, 1800m		yes (Based on Schubotz 1912)	0.424544	29.79893
ZMB	77536	Rwanda	Nyungwe-Waterfall trail			-2.4482	29.111267
ZMB	78947	Rwanda	Gishwati Forest			-1.763027	29.37149
ZMB	78948	Rwanda	Gishwati Forest			-1.763027	29.37149
ZMB	78950	Rwanda	Nyungwe-Rwasenkoko (Uwasenkoko)			-2.524033	29.353117
ZMB	78951	Rwanda	Nyungwe-Rwasenkoko (Uwasenkoko)			-2.524033	29.353117
ZMB	78952	Burundi	Bururi Forest Reserve			-3.934367	29.619167
ZMB	78953	Burundi	Bururi Forest Reserve			-3.934367	29.619167
ZMB	78954	Burundi	Bururi Forest Reserve			-3.934367	29.619167
ZMB	78955	Burundi	Bururi Forest Reserve			-3.934367	29.619167
ZMB	78956	Rwanda	Nyungwe-near Pindura			-2.476817	29.228411
	uncollected	Rwanda	Nyungwe-Nshili			-2.79235	29.4133
	uncollected	Rwanda	Nyungwe-Kamiranzovu			-2.483067	29.152033
	uncollected	Rwanda	Nyungwe-western park boarder			-2.463833	29.1008

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