



A new species of *Hyperolius* Rapp, 1842 (Anura: Hyperoliidae) from the Serra da Chela mountains, south-western Angola

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Abstract

A new species of African reed frog (genus *Hyperolius* Rapp, 1842) is described from a high altitude, forested gorge in the Serra da Chela mountain range near the village of Humpata, Lubango area, Huila Province, south-western Angola. It is currently only known from its type locality. Phylogenetic reconstruction using the mitochondrial 16S marker reveals that it is the sister taxon of the poorly known *Hyperolius cinereus* (>4% sequence divergence) also described from Angola, and forms part of a larger clade comprising *H. platyceps*. The new species is distinguished from other closely related *Hyperolius* species by genetic divergence, vocalisation, an unusual torrent stream habitat, and colouration.

Key words: Amphibian, Hyperoliidae, *Hyperolius*, Reed frog, Biodiversity, Afrotropical, Angola

Introduction

The amphibian fauna of Angola is poorly known, and a recent summary listed 86 species within 7 families and 20 genera, of which 14 species (16%) were considered endemic (Blanc & Fretey 2000). Frost (2011) listed 99 amphibian species for Angola, although this contained a number of taxa (e.g. *Cardioglossa leucomystax*) that were presumed to occur, but whose presence remains unconfirmed. Despite these discrepancies, amphibian diversity in Angola is considerably lower than that of adjacent Democratic Republic of Congo (DRC) to the north (ca. 232 species (Table 2 in Poynton 1998, Frost 2011), but comparable to the Zambesiaca area (which includes Botswana, Caprivi [Namibia], Zambia, Malawi, Mozambique and Zimbabwe: 113 species, Poynton & Broadley 1991).

The genus *Hyperolius* Rapp, 1842 is the most speciose African amphibian genus (Poynton 1999), with more than 120 recognised species (Frost 2011). Frost (2011) further lists 31 *Hyperolius* species known from Angola, of which 7 species are recorded from the Cabinda enclave, which is politically part of Angola but lies to the north of the Congo River and shows greater affinities to DRC biodiversity. Moreover, two species listed by Frost (2011), i.e. *H. obscurus* and *H. poweri*, have not been recorded from Angola, including Cabinda. The taxonomic status and evolutionary relationships of many Angolan hyperoliid species are equivocal, e.g. *Hyperolius nasutus* complex (Channing *et al.* 2002; Schiøtz 2006), whilst others await rediscovery or reassessment since their original description between 50 and 135 years ago (e.g. *Hyperolius bicolor* Ahl, 1931; *H. cinereus* Monard, 1937; *H. fasciatus* (Ferreira, 1906); *H. ferreirai* Noble, 1924; *H. fuscigula* Bocage, 1866; *H. gularis* Ahl, 1931 and *H. vilhenai* Laurent, 1964). The latest phylogeny of African hyperoliids (Schick *et al.* 2010) emphasized difficulties in *Hyperolius* taxonomy resulting from the generally poor external diagnostic characters and substantial intraspecific colour and pattern variation. This, together with the often inadequate original descriptions, as for example those of Ahl (1924), has resulted in considerable taxonomic confusion.

Due to the protracted civil war in Angola (1975–2002) opportunities for scientific research in the region were limited. As a consequence, knowledge of national biodiversity remains poor relative to that of southern and eastern Africa, particularly that of the herpetofauna. The early studies of Bocage in the late 19th century (see summary in Bocage 1895) laid the groundwork for herpetology in the country, followed by regional collections described by Monard (1931, 1937), Schmidt (1933), Parker (1936), Mertens (1938), Hellmich (1957a, b) and FitzSimons (1959). Laurent's studies (1952, 1954, 1964), based on collections in the Dundo Museum in Lunde Norte Province, resulted in the description of numerous new reptiles and amphibians from Angola and adjacent DRC. Subsequently, the onset of protracted hostilities in the region severely hampered scientific collecting, with only a few opportunistic collections (e.g. Branch & McCartney 1992) and the description of few new Angolan species (e.g. Haacke 2008: *Afrogecko plumicaudus*, Poynton & Haacke 1993: *Poyntonophrynus grandisonae* and one species of *Dalophia* and two species of *Monopeltis*: Gans 1976), all based on specimens collected before civil war erupted.

As part of the Angolan Biodiversity Assessment Capacity Building Project, Biodiversity Rapid Assessment surveys were undertaken in January 2009 to the south-western provinces (Huila and Namibe) (Huntley 2009), and in May 2011 to Lagoa Caurumbo in Lundu Norte Province, north-eastern Angola (Huntley 2011; Fig. 1). During these surveys detailed herpetological collections from diverse habitats were made. In total, six different species of *Hyperolius* were collected, most of which could be assigned to species level. Among the collected specimens were two adults and several tadpoles of an unusual *Hyperolius* discovered in a narrow, forested gorge at high altitude in the Serra da Chela mountain range near Humpata, Lubango area, Huilia Province (Fig. 1). Various features of the colouration, morphology, habitat, vocalisation, calling position, and level of genetic divergence all indicate that these individuals are not referable to any known taxon, and we therefore take this opportunity to describe them as a new species. Utilising a combination of new and previously published sequences of the 16S mitochondrial marker, we also examine the relationship of this new reed frog to other species in the genus *Hyperolius*.

Material and methods

Sampling. *Hyperolius* specimens for this study were collected on the escarpment near Humpata, Huila Province, south-western Angola in 2009 and Lagoa Carumbo, Lundu Norte Province, northern Angola, in 2011 (Fig. 1). Specimens were formalin-fixed for 48h, and transferred to 50% isopropanol for long-term storage in the herpetological collection of the Port Elizabeth Museum (PEM), with representative material to be returned to Angola to form part of the revitalized faunal collections of the Lubango Museum (Huntley 2009). Prior to formalin fixation, tissue samples from thigh muscle (adults) or tail clipping (tadpoles) were taken, and stored in 99% ethanol (Table 1).

TABLE 1. Species localities, museum accession numbers, and GenBank accession numbers of the specimens used in this study. * denotes individuals sequenced for this study. N/A indicates no corresponding information available.

Species	Museum/Field Accession	Locality	GenBank Accession
<i>Hyperolius angolensis</i>	PEM A10106	Humpata, Angola	JQ513623*
<i>Hyperolius angolensis</i>	PEM A10024	Lagoa Carumbo, Angola	JQ513626*
<i>Hyperolius angolensis</i>	PEM A10025	Lagoa Carumbo, Angola	JQ513625*
<i>Hyperolius angolensis</i>	N/A	Namibia or Botswana	AF282411
<i>Hyperolius acuticeps</i>	NMK A/3929/2	Runda-Gigiri, Kenya	FJ594074
<i>Hyperolius alticola</i>	CAS 207322	Bioko, Equatorial Guinea	AY603984
<i>Hyperolius baumanni</i>	N/A	Apesokubi, Ghana	FJ594075
<i>Hyperolius benguellensis</i>	PEM A10107	Humpata, Angola	JQ513638*
<i>Hyperolius bobirensis</i>	ANK101	Ankasa, Ghana	GU443982
<i>Hyperolius castaneus</i>	CAS 202372	Ruhizha, Uganda	FJ151059
<i>Hyperolius chlorosteus</i>	MOR S01.55	Mont Sangbé NP, Ivory Coast	FJ594076
<i>Hyperolius chelaensis</i> sp. nov. (Holotype)	PEM A9223	Serra da Chela gorge, Angola	JQ513627*

continued next page

TABLE 1. (continued)

Species	Museum/Field Accession	Locality	GenBank Accession
<i>Hyperolius chelaensis</i> sp. nov. (Paratype)	PEM A9224	Serra da Chela gorge, Angola	JQ513628*
<i>Hyperolius chelaensis</i> sp. nov. (Tadpole)	PEM T350	Serra da Chela gorge, Angola	JQ513629*
<i>Hyperolius cinereus</i> (northern)	PEM A10049	Lagoa Carumbo, Angola	JQ513632*
<i>Hyperolius cinereus</i> (northern)	PEM A10055	Lagoa Carumbo, Angola	JQ513633*
<i>Hyperolius cinereus</i> (northern)	PEM A10041	Lagoa Carumbo, Angola	JQ513634*
<i>Hyperolius cinereus</i> (southern)	PEM A9212	Serra da Chela plateau, Angola	JQ513635*
<i>Hyperolius cinereus</i> (southern)	PEM A9202	Serra da Chela plateau, Angola	JQ513636*
<i>Hyperolius cinereus</i> (southern)	PEM A9210	Serra da Chela plateau, Angola	JQ513637*
<i>Hyperolius cinnamomeoventris</i>	ZFMK 73207	Kinguélé, Gabon	FJ594077
<i>Hyperolius cinnamomeoventris</i>	CAS 202493	Bwindi, Uganda	AY603985
<i>Hyperolius cinnamomeoventris</i>	AC 3008	Kalandula, Angola	HM064461
<i>Hyperolius cinnamomeoventris</i>	AC 3017	Kalandula, Angola	GU443990
<i>Hyperolius concolor</i>	N/A	Haute Dodo, Ivory Coast	FJ594078
<i>Hyperolius cystocandicans</i>	ZFMK 77611	Mt. Kenya, Kenya	FJ594079
<i>Hyperolius frontalis</i>	CAS 201986	Bwindi NP, Uganda	AY603986
<i>Hyperolius fusciventris</i>	N/A	Haute Dodo, Ivory Coast	FJ594080
<i>Hyperolius glandicolor</i>	NMK A/3862/1	Aberdares, Kenya	FJ594081
<i>Hyperolius guttulatus</i>	MB 20267	Mt. Doudou, Gabon	FJ594082
<i>Hyperolius kivuensis</i>	SL471	Bundibugyo, Uganda	GU443979
<i>Hyperolius kivuensis</i>	NMK A/3867/4	Kakamega, Kenya	AY323919
<i>Hyperolius laurenti</i>	ANK72	Ankasa, Ghana	GU443987
<i>Hyperolius lateralis</i>	NMK A/3925/1	Kakamega, Kenya	AY323924
<i>Hyperolius mosaicus</i>	ZFMK 73140	Mt. Cristal, Gabon	AY323923
<i>Hyperolius</i> cf. <i>nasutus</i>	PEM A10059	Lagoa Carumbo, Angola	JQ513639*
<i>Hyperolius</i> cf. <i>nasutus</i> (tadpole)	PEM T490	Village Capaia, Angola	JQ513640*
<i>Hyperolius nasutus</i>	N/A	Comoé NP, Ivory Coast	FJ594086
<i>Hyperolius ocellatus</i>	CAS 207321	Bioko, Equatorial Guinea	AY603988
<i>Hyperolius pardalis</i>	ZFMK73153	Mt. Cristal, Gabon	AY323922
<i>Hyperolius picturatus</i>	N/A	Haute Dodo, Ivory Coast	FJ594090
<i>Hyperolius platyceps</i>	MB5181	Gabon	FJ594091
<i>Hyperolius riggenbachi</i>	MVZ234752	Ndop, Cameroon	GU443976
<i>Hyperolius</i> cf. <i>steindachneri</i>	PEM A10028	Lagoa Carumbo, Angola	JQ513631*
<i>Hyperolius</i> cf. <i>steindachneri</i>	PEM A10029	Lagoa Carumbo, Angola	JQ513630*
<i>Hyperolius sylvaticus</i>	BOB37	Ivory Coast	GU443981
<i>Hyperolius torrentis</i>	MOR G62	Kyabobo NP, Ghana	FJ594093
<i>Hyperolius tuberculatus</i>	ZFMK 73144	Mt. Cristal, Gabon	AY323921
<i>Hyperolius viridiflavus</i>	ZFMK 66718	Little Brak, South Africa	AF215441
<i>Hyperolius zonatus</i>	N/A	Tai NP, Ivory Coast	FJ594096
Outgroup			
<i>Kassina senegalensis</i>	N/A	St. Lucia, South Africa	AF215445
<i>Kassina maculata</i>	ZFMK 66445	Kwambonambi, South Africa	AF215444
<i>Afrivalus delicatus</i>	ZFMK 68792	Kwambonambi, South Africa	AF215428
<i>Acanthixalus spinosus</i>	ZFMK 72000	Nyasoso, Cameroon	AF215427

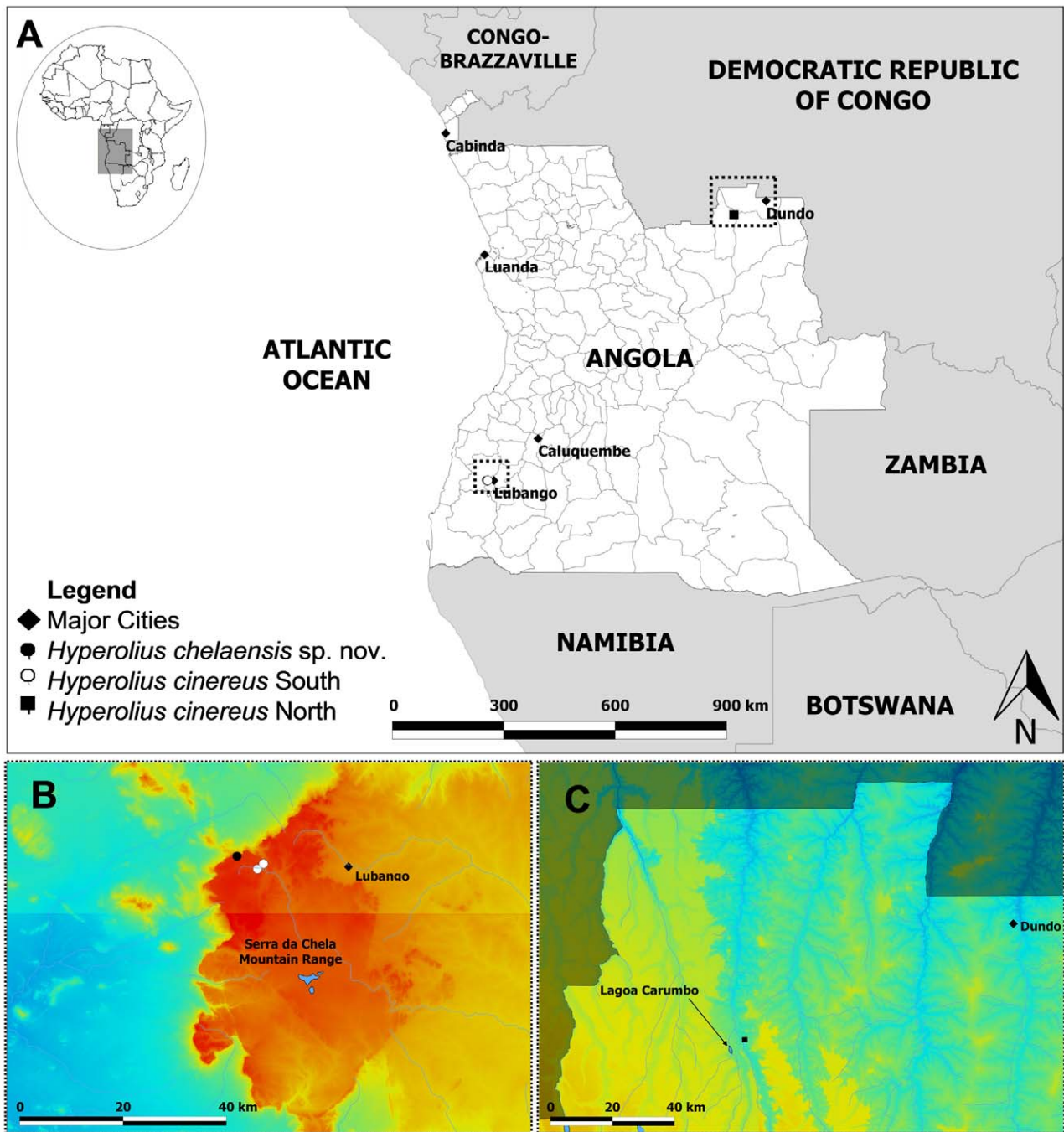


FIGURE 1. Map showing the collection locality of *Hyperolius chelaensis* sp. nov. and both populations of *H. cinereus* (Caluquembe is the type locality of *H. cinereus* Monrad, 1937 and Dundo is the collection locality on which Laurent (1964) based his *H. cinereus* description). Insert B and C represents the bracketed areas in A, colour represents altitude (red—high, blue—low).

Measurements and calls. The following morphological features were recorded from the preserved adult specimens: snout-vent length (SVL), head width (HW), interorbital distance (IOD), eye-nostril distance (END), nostril-snout tip distance (NSD), eye diameter (ED), thigh length (THL), tibia length (TL), foot length (FL). All measurements were taken with digital callipers to 0.05 mm precision with the aid of a stereo microscope and rounded off to the first decimal place. Webbing formulae followed the scheme of Rödel (2000). Morphological descriptions and labial tooth row formulae (LTRF) in general follows McDaiarnid & Altig (1999) and Altig (2007). Tadpole developmental stages are based on Gosner (1960). The following measurements were taken of tadpoles to the nearest 0.05 mm under a dissecting microscope with digital callipers and rounded off to the first decimal place. tTL, total length (distance from snout to tail tip); BL, body length (distance from snout to body-tail junction, a coronal line

that abuts the caudal edge of the body); TAL, tail length (tTL-BL); BH, maximum body height; BW, maximum body width; TMW, basal tail muscle width; MTH, maximum tail height; IOD, inter-orbital distance (measured from centres of orbits); IND, inter-narial distance (measured from centres of narial apertures); NOD, nasal orbital axis (measured perpendicular from IND to IOD); SND, snout to nostril axis (measured from tip of snout to the perpendicular of the IND); SOD, snout to orbital axis (NOD+SND); SS, snout to spiracle (measured on a line parallel to the longitudinal axis of the body); ED, eye diameter; ND, narial diameter; and ODW, oral disc width (the widest transverse distance). Advertisement calls were recorded in the field using an Olympus Voice Recorder VN-3500PC with a Sony F-V4T Microphone and analyzed using Raven Lite version 1.0 (Cornell Lab of Ornithology).

DNA extraction, amplification and sequencing. Total genomic DNA was extracted with a proteinase-K digestion followed by a salt extraction (Bruford *et al.* 1992). One mitochondrial (16S) marker was amplified using the following PCR conditions: 2 µl genomic DNA (25ng/µl) was added to a reaction containing 10x thermophilic buffer (50mM KCl, 10mM Tris-HCl, pH 9.0), 2.5mM MgCl₂, 0.2µM of each primer, 0.2mM dNTPs and 0.025U/l Taq polymerase. The PCR profile included an initial denaturing step at 95 °C for 1 min, followed by 35 cycles of 95 °C for 1 min, 55 °C for 30sec, and 72 °C for 60 sec, with a final extension at 72 °C for 5 min. Annealing temperatures varied between 52–57°C, using primers 16Sa and 16Sb (Palumbi *et al.* 1996). PCR products were sent for sequencing to Macrogen, Inc. (Korea), using the forward primer. Sequences were checked and aligned using Geneious Pro v.4.5.6 (Drummond *et al.* 2009) and saved as nexus files. All new sequences have been accessioned into GenBank (Table 1).

Phylogenetic analysis. A total of 56 sequences of the 16S mitochondrial marker from the genus *Hyperolius* were included in a phylogenetic analysis, 37 of which were already published in GenBank (Table 1). Sequences from three (two adults and one tadpole) individuals of the unidentified taxon from the Serra da Chela forest gorge were included, as were sequences from three individuals referable to *H. cinereus* Monard, 1937, from a nearby stream on the plateau above the gorge (Fig. 1). As a comparison, sequences from three other specimens referable to *H. cinereus* (cf. Laurent 1964) from Lagoa Carumbo, northern Angola were also included. The following species were included as outgroup taxa: *Kassina senegalensis*, *K. maculata*, *Afrixalus delicatus* and *Acanthixalus spinosus* (Schick *et al.* 2010, Veith *et al.* 2009). A Bayesian analysis was conducted on the 16S data set (560 base pairs, with 68 bases excluded due to ambiguous alignment) using MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003) via remote upload at the CBSU cluster (cbsuapps.tc.cornell.edu). The model included a single partition with 6 rate categories, based on a preliminary examination of the dataset using Modeltest 3.6 (Posada & Crandall 1998), where the GTR+I+G was indicated as the best model to fit the dataset. The MCMC was run in parallel for 10 million generations and trees sampled every 1,000 generations. The first 5 million generations (5,000 trees) were removed as burn-in after examination of the average standard deviation of split frequencies (< 0.005), the convergence diagnostic (PSRF values ~ 1.0) as well as the log-probabilities and the values of each parameter for stabilisation. The ESS values were checked for all parameters using Tracer v1.4.1 (Rambaut & Drummond 2007) to ensure the effective sample sizes exceeded 200. The remaining 5,000 trees were used to construct a 50% majority rule tree and nodes with ≥ 0.95 posterior probability were considered supported. In addition, a maximum likelihood (ML) search was run in GARLI (Zwickl 2006), with all parameters estimated, and a random starting tree. This analysis was run three times to ensure that independent ML searches produced the same topologies. Nodes with a bootstrap value of $\geq 70\%$ were considered supported in this analysis.

Results

Hyperolius chelaensis sp. nov.

Chela Mountain reed frog

(Figs 5, 6 & 7)

Holotype. An adult male (PEM A9223; Fig. 5) deposited in the Port Elizabeth Museum (PEM), South Africa. Collected 14 January 2009, by W. Conradie from a small patch (< 2.5ha.) of Afromontane forest in a small gorge draining from Serra da Chela above the Estacao Zootecnica, near Humpata, Lubango, Angola (14°53'22" S; 13°16'27" E, 1413CD, 2045 m above sea level). Tissue has been preserved under the field number KTH09-049 at the South African Biodiversity Institute (SANBI), Kirstenbosch, South Africa.

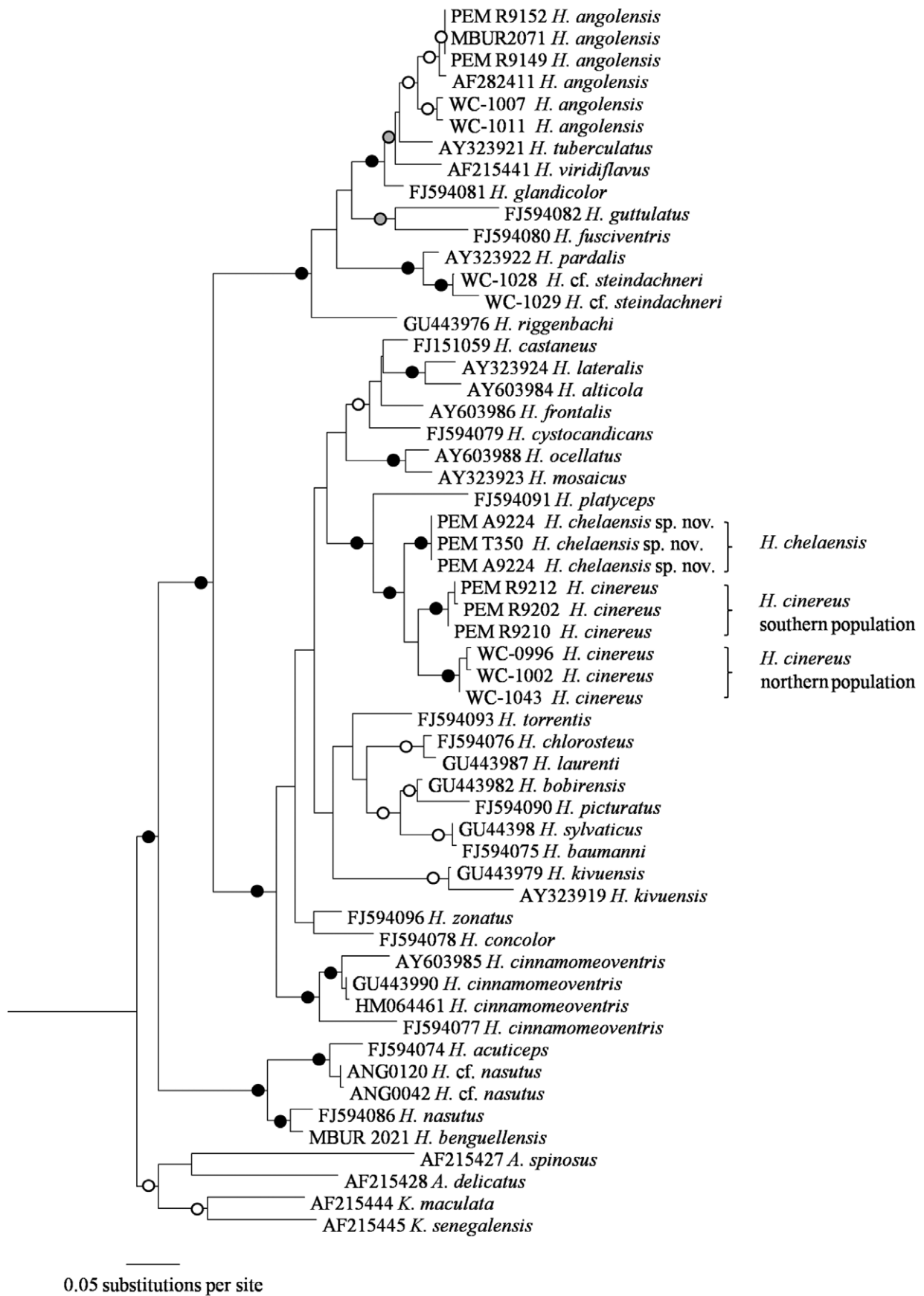


FIGURE 2. Maximum likelihood topology for *Hyperolius*. Black circles represent nodes supported by Bayesian posterior probabilities (≥ 0.95) and ML bootstrap ($> 70\%$), grey circles represent nodes supported by Bayesian posterior probabilities only, and white circles represent nodes supported by ML bootstrap only.

TABLE 2. Pairwise uncorrected net p-distances (16S marker) between species of selected *Hyperolius* which represent major clades included in the phylogeny for this study.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
1 <i>angolensis</i>																						
2 <i>ocellatus</i>	0.126																					
3 <i>lateralis</i>	0.131	0.084																				
4 <i>frontalis</i>	0.117	0.077	0.067																			
5 <i>castaneus</i>	0.139	0.073	0.061	0.048																		
6 <i>chlorosteus</i>	0.153	0.105	0.107	0.107	0.101																	
7 <i>cystocandicans</i>	0.129	0.074	0.075	0.061	0.056	0.093																
8 <i>fusciventris</i>	0.089	0.136	0.147	0.138	0.146	0.150	0.140															
9 <i>grandicolor</i>	0.037	0.128	0.135	0.119	0.136	0.144	0.121	0.082														
10 <i>platyceps</i>	0.161	0.097	0.111	0.106	0.101	0.123	0.110	0.166	0.155													
11 <i>torrentis</i>	0.155	0.088	0.097	0.099	0.089	0.070	0.094	0.147	0.145	0.093												
12 <i>xonatus</i>	0.131	0.084	0.084	0.081	0.068	0.081	0.076	0.131	0.120	0.107	0.073											
13 <i>bobirensis</i>	0.150	0.096	0.095	0.096	0.094	0.070	0.098	0.154	0.134	0.105	0.073	0.084										
14 <i>cinnamomeoventris</i>	0.115	0.083	0.076	0.080	0.077	0.075	0.075	0.126	0.115	0.082	0.069	0.052	0.078									
15 <i>kiwensis</i>	0.127	0.096	0.102	0.100	0.090	0.103	0.101	0.132	0.129	0.106	0.085	0.078	0.086	0.076								
16 <i>riggenbachi</i>	0.087	0.143	0.136	0.128	0.143	0.147	0.134	0.111	0.085	0.161	0.148	0.137	0.127	0.110	0.135							
17 <i>sylvaticus</i>	0.150	0.098	0.106	0.106	0.111	0.089	0.100	0.160	0.145	0.109	0.078	0.096	0.042	0.081	0.099	0.131						
18 <i>cf. steindachneri</i>	0.106	0.140	0.145	0.138	0.138	0.161	0.140	0.094	0.091	0.147	0.165	0.153	0.154	0.124	0.132	0.111	0.164					
19 <i>nasutus</i>	0.155	0.156	0.166	0.150	0.154	0.165	0.153	0.143	0.136	0.155	0.153	0.159	0.160	0.138	0.139	0.157	0.177	0.151				
20 <i>cineurus</i> (south)	0.137	0.076	0.095	0.082	0.083	0.102	0.088	0.131	0.135	0.081	0.093	0.089	0.097	0.076	0.108	0.138	0.107	0.137	0.151			
21 <i>cineurus</i> (north)	0.155	0.070	0.099	0.089	0.086	0.106	0.094	0.132	0.142	0.078	0.089	0.090	0.103	0.085	0.103	0.151	0.109	0.139	0.154	0.041		
22 <i>chelaensis</i> sp. nov.	0.147	0.091	0.099	0.092	0.086	0.120	0.101	0.138	0.148	0.077	0.092	0.094	0.098	0.072	0.094	0.139	0.106	0.134	0.151	0.045	0.042	

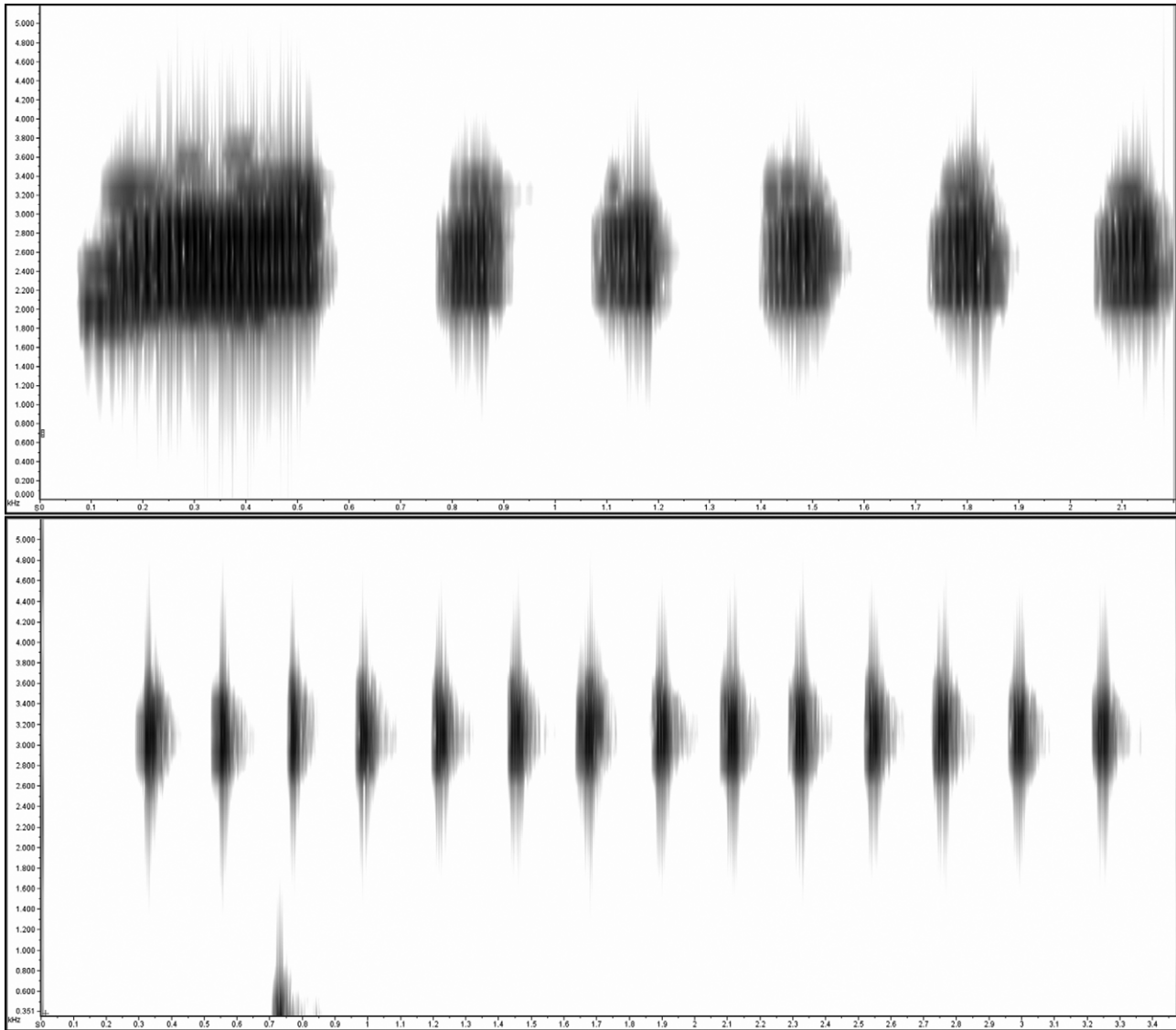


FIGURE 3. Spectrogram of the advertisement calls of *Hyperolius chelaensis* **sp. nov.** (above) and *H. cinereus*, southern population (below). X-axis indicates time in seconds (s) and y-axis indicates frequency in kilohertz (kHz).

Paratype. An adult female (PEM A9224; Fig. 6), collection data same as holotype. Tissue has been preserved under the field number KTH09-050 at SANBI.

Diagnosis. A species referred to *Hyperolius* due to the following characters: pupil horizontal; vocal sac present in male, with the gular flap oval with lateral and posterior margins free; terminal discs on fingers and toes expanded, rounded; tympanum hidden; skin smooth (Schjøtz 1999; Channing 2001); and molecular monophyly for the 16S mitochondrial marker with other *Hyperolius* taxa.

The new species can be distinguished from other *Hyperolius* in Angola and adjacent regions by: ventrum uniform yellow without darker pigmentation (heavily pigmented in *H. steindachneri*); pale canthal and dorsolateral lines (red in *H. kachalolae*; dark in *H. kivuensis*, *H. cinereus*, *H. vilhenai*, and *H. bocagei*); no pale triangle on snout (present in *H. ocellatus*); no light heel spot (present in *H. mitchelli*); no translucent green belly skin (present in *H. nasutus* complex); a single dorsolateral band present and no dorsal patterns (distinct from quite variable patterns in *H. major*, *H. pictus* and the *H. angolensis* complex); a single dorsolateral band (at least two in *H. quinquevittatus*); no distinct separation between dorsal ventral colouration (distinctly bicolor pattern with light dorsolateral line in *H. cinnamomeoventris*, *H. cinereus*); throat without spines (spinose in *H. spinigularis*).

In the phylogenetic analysis (see Results) it is sister to *H. cinereus*, and this clade in turn is sister to *H. platyceps*. The new species differs from *H. platyceps* by not having a dorsal hourglass pattern; a smaller gular flap; and no light triangle on snout. In addition it has a different habitat (cascade stream vs bushland) and advertisement call

(burst of calls vs a single clack). From *Hyperolius cinereus* it differs in not having: a brightly coloured (uniform green to bluish) dorsum; bright red inner thighs; black longitudinal stripes in males; and large yellow protruding eyes. It further differs in having a lower frequency advertisement call (an initial creak and a burst of 4–5 short notes compared with a train of 14–16 notes in *H. cinereus*); having a cathal or dorsolateral line; and larger size (male 24.6 mm vs 20.2 mm; female 31.5 mm vs 24.1 mm). It finally differs in habitat preference (Afromontane forest stream vs grassland).

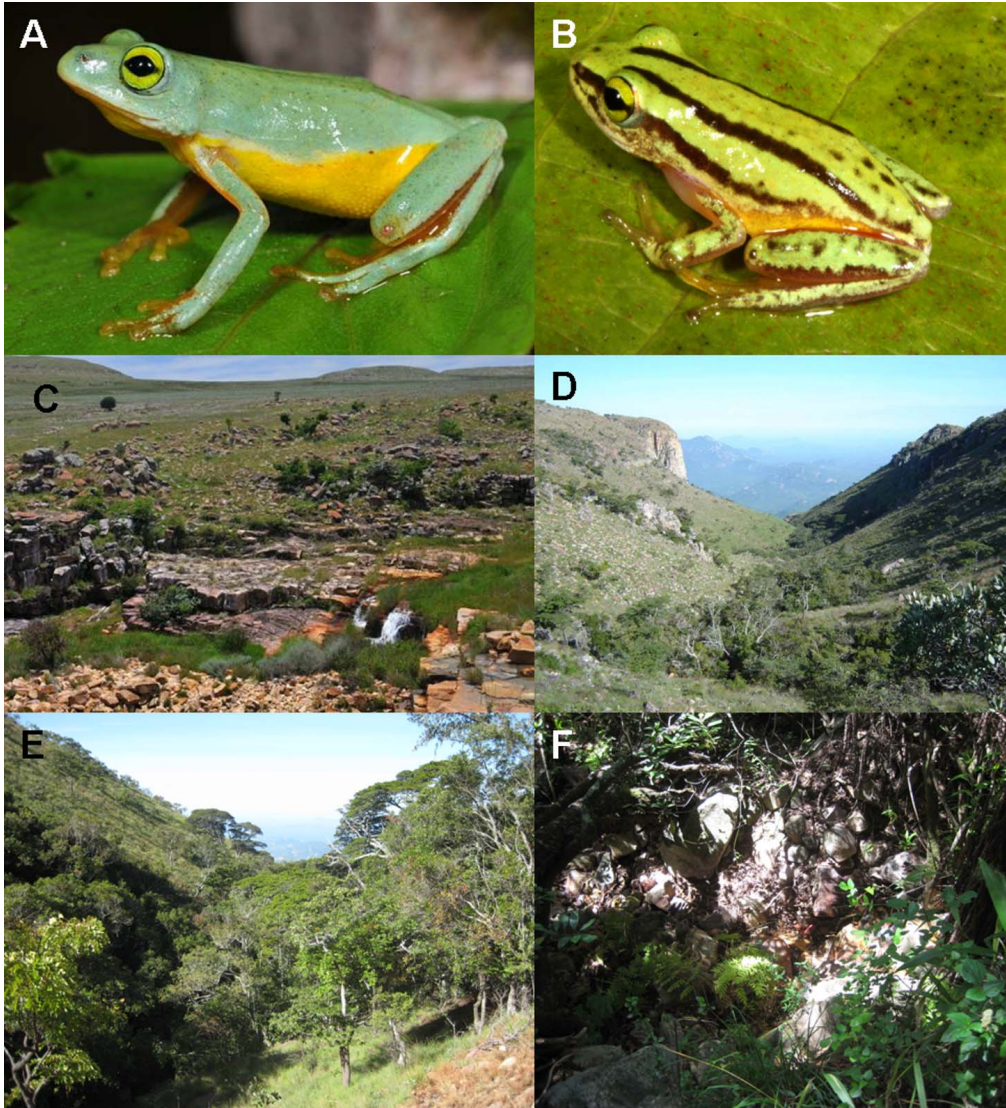


FIGURE 4. A—Adult female *Hyperolius cinereus* from plateau above Estacao Zootechnica, near Humpata, B—Adult male *H. cinereus* from Lagoa Carumbo, C—habitat of *H. cinereus*, D to F—habitat of *H. chelaensis* **sp. nov.**

Description of the Holotype. An adult male measuring 24.6 mm snout-vent length; medium size *Hyperolius* with short head and rounded snout, HL 31.9 % of SVL; habitus squat; head-width directly behind eyes: 7.5 mm; small gular flap present; eyes not protruding, eye-diameter: 3.1 mm; pupil horizontal to circular; distance eye-nostril; 2.3 mm; distance nostril-snout tip: 0.9 mm, nostril almost three times closer to snout than eye; tympanum absent; thigh length: 10.5 mm; tibia similar length to thigh; foot including longest toe IV: 16.6 mm; tip of fingers and toes with enlarged discs; webbing between fingers minimal, only reaching proximal subarticular tubercle and absent between first and second finger; inner metatarsal tubercle present, but small and indistinct; subarticular tubercles on fingers well developed; feet well webbed with webbing formula: 1 (0.25); 2 i/e (1-0.25); 3 i/e (1-0.25); 4 i/e (1); 5 (0.25), toe formula: 4>5>3>2>1, finger formula: 3>4>2>1; dorsal skin smooth; ventral skin faintly granular. Further body dimensions and ratios are summarised in Table 3.

TABLE 3. Morphological features (mm) and ratios of *Hyperolius chelaensis* **sp. nov.** and *Hyperolius cinereus*. See material and methods for explanation of abbreviations.

	<i>H. chelaensis</i> sp. nov.		<i>H. cinereus</i> (Male n = 33)		<i>H. cinereus</i> (Female n = 7)	
	Holotype PEM A9223	Paratype PEM A9224	Mean	Standard Deviation	Mean	Standard Deviation
SVL	24.6	31.5	20.2	1.3	24.1	2.9
HW	7.5	8.9	5.9	0.3	6.8	0.8
ED	3.1	3.2	2.4	0.2	2.7	0.4
END	2.3	2.6	1.8	0.2	2.2	0.4
NSD	0.9	1.1	1.3	0.2	1.2	0.3
IOD	2.8	3.7	2.5	0.2	2.7	0.2
IND	2.5	2.5	1.8	0.1	2.1	0.2
THL	10.5	14.6	9.4	0.7	11.2	1.0
TL	10.9	13.7	9.4	0.7	11.0	0.9
FL	16.6	21.1	13.6	1.0	16.7	1.8
HW/SVL	0.3	0.3	0.3		0.3	
END/HW	0.3	0.3	0.3		0.3	
NSD/ED	0.3	0.3	0.6		0.4	
ED/TL	0.3	0.2	0.3		0.2	
END/IOD	0.8	0.7	0.7		0.8	
THL/SVL	0.4	0.5	0.5		0.5	
TL/SVL	0.4	0.4	0.5		0.5	
FL/SVL	0.7	0.7	0.7		0.7	

Dorsum light brownish in colouration with white speckles (Fig. 5); iris golden colour; uniform yellow ventrum, throat and feet; yellow colour of ventrum faded in alcohol to a light beige; no dark lateral pigmentation; red coloured inner thighs; tip of fingers and toes yellow; faint light coloured dorsolateral line extending from the tip of snout through the eye and fading away at mid-body.

Description of the paratype. The paratype (female) measured 31.5 mm and in general resembles the holotype. It differs in that its dorsum is darker in colouration and the dorsolateral stripe does not extend much further forward than the eyes. Sexual dimorphism follows that of other *Hyperolius* species, eg. absence of vocal sac and gular flap in females.

Description of tadpole. Based on two lots of tadpoles (n=5) (PEM T350–351; Fig. 7) which were collected at the same locality as the adult types by W. Conradie and G.J. Measey. Tissue has been preserved under the field number KTH09-074 at SANBI. Sequence divergence between the holotype and tadpole was 0% indicating that the tadpoles were conspecific with the adults. Tadpoles varied between Gosner stage 25 and 26. *Lateral view* (Fig. 3A): Body round to ovoid; body width nearly equal to the body height; snout oblique; mouth directed ventrally; nasals rounded, very small, positioned laterally, closer to snout than to pupils; eyes moderately sized (ED 9% of BL), positioned and directed laterally; spiracle sinistral, tubular, small, joined to body wall, positioned laterally and situated closer to snout than vent (SS 44% of BL), oval opening directed upwards at a 45° angle, at the height of the middle of the lower part of the caudal muscle; tail musculature moderate (TMH 66% of BH and 70% of MTH), tapering gradually to round tail tip; tail fins of moderate size, deepest point two thirds down tail; upper fin not extending onto body, slightly convex to the end of the tail; lower fin slightly convex to the end of tail. *Dorsal view* (Fig. 3B): Body oval, wider than high, widest just behind the spiracle opening; snout rounded; nasals widely spaced; interorbital distance nearly equal to internarial distance; tail muscle width quarter of body width. *Ventral view* (Fig. 3C): Eyes bulging and not visible in ventral view; vent positioned supramarginal, dextral and short with an oval opening. **Oral disc:** Positioned and directed ventrally, moderately small (ODW 26% of BW); LTRF: 1/3(1), third posterior row 75% the length of the first row; jaw sheets moderately pigmented, capsulated and rounded;

lateral process short; single row of posterior marginal papillae, and a single row of anterior marginal papillae; dorsal gap free of papillae (up to 75% free); posterior corners with a few submarginal papillae; lateral margins of oral disc not indented. **Colouration pattern:** Body dark brown-orange with randomly scattered dark spots, ventrum paler with scattered darker pigmentation, internal collided intestine visible through skin; tail musculature more heavily pigmented than fins, particularly on the ventral and dorsal margins; dark axial stripe present on tail musculature; fins mottled; terminal half of two tadpoles (PEM T351) dark. **Size:** Total length 30.1–45.6 mm and body length 12.2–18.9 mm. The tail is 140% of the body length, and 59% of tTL. Table 4 summarises all measurements.

TABLE 4. Morphological features (mm) of *Hyperolius chelaensis* sp. nov. tadpole. See material and methods for explanation of abbreviations.

	#1*	#2**	#3	#4	#5	Average	Standard Deviation
Gosner stage	25	26	25	26	26		
Total Length (tTL)	38.9	NA	30.1	45.6	43.2	39.5	6.8
Body Length (BL)	18.0	18.9	12.2	17.6	16.8	16.7	2.6
Tail Length (tTL-BL)	20.9	NA	17.9	28.1	26.4	23.3	4.7
Body Height (BH)	8.5	9.3	5.6	6.7	6.4	7.3	1.5
Body Width (BW)	10.1	11.4	6.1	9.5	7.7	9.0	2.1
Tail Muscle Width (TMW)	2.1	2.6	1.6	2.4	2.4	2.2	0.4
Tail Height (MTH)	5.9	7.6	5.6	7.9	7.5	6.9	1.1
Tail Muscle Height (TMH)	5.2	5.7	3.2	5.4	4.3	4.8	1.0
Interorbital Distance (IOD)	4.0	4.7	3.7	4.3	4.6	4.3	0.4
Internarial distance (IND)	3.7	4.1	2.9	3.7	4.0	3.7	0.5
Nasal orbital axis (NOD)	2.8	3.1	2.3	3.1	3.0	2.8	0.3
Snout to nostril axis (SND)	2.2	2.0	1.7	2.0	2.2	2.0	0.2
Snout to orbital axis (SND+NOD)	4.9	5.1	4.0	5.0	5.1	4.8	0.5
Snout to Spiracle (SS)	7.2	8.9	5.4	8.1	7.6	7.4	1.3
Eye diameter (ED)	1.4	2.0	1.2	1.7	1.8	1.6	0.3
Narial diameter (ND)	0.3	0.4	0.3	0.3	0.3	0.3	0.0
Oral disc width (ODW)	2.4	2.4	1.9	2.4	2.4	2.3	0.2
LTRF	1/3(1)	1/3(1)	1/3(1)	1/3(1)	1/3(1)		

* illustrated tadpole; **sequenced specimen, tip of tail used as genetic sample.

Advertisement call. The advertisement call was recorded on 14 January 2009, at 10h45 at an air temperature of approximately 25°C. Recordings were made from the holotype male before capture, and are deposited in PEM. The call consists of two parts; an initial creak of 0.4–0.5 seconds, followed by a burst of four to five short chirps each of about 0.1 seconds in duration and separated by an interval of 0.25–0.30 seconds, and with an emphasis frequency between 2400–2700 Hz (Fig. 3).

Distribution and habitat. The species was found inhabiting a cascade stream habitat in a small patch of Afromontane forest draining north from the Serra da Chela (Fig. 4D–F). The only other amphibian observed sharing the same habitat was *Amietia angolensis*. Additional surveys are required to determine whether *H. chelaensis* sp. nov. occurs elsewhere in similar habitat along the Chela and Leba mountain ranges (Fig. 1).

Natural history. Tadpoles of *H. chelaensis* sp. nov. were found in slow flowing gullies of the main stream with substrate of leaf litter and small pebbles. All tadpoles collected were in early developmental stages in January, near the end of the rainy season (which commences in November). Although *Hyperolius cinereus*, *H. benguellensis*

and *H. angolensis* were heard calling in the region, no eggs or tadpoles of these species were found at the site. This may indicate that *H. chelaensis* **sp. nov.** breeds later than conspecifics, perhaps for the small tadpoles to avoid floodwaters at the height of the rainy season. The tadpoles do not appear to be adapted for torrent streams. The male was calling half-submerged and flat against a rock in slow flowing water. This is an unusual calling position for hyperoliids and has not been observed in any other *Hyperolius* species. Although *H. torrentis* has been observed calling near waterfalls or flowing water in forested areas, this was from emergent vegetation and not submerged in water (Schjötz 1967, Rodel & Agyei 2003).

Etymology. The species is named after the mountain range, Serra da Chela, from which it is currently only known.

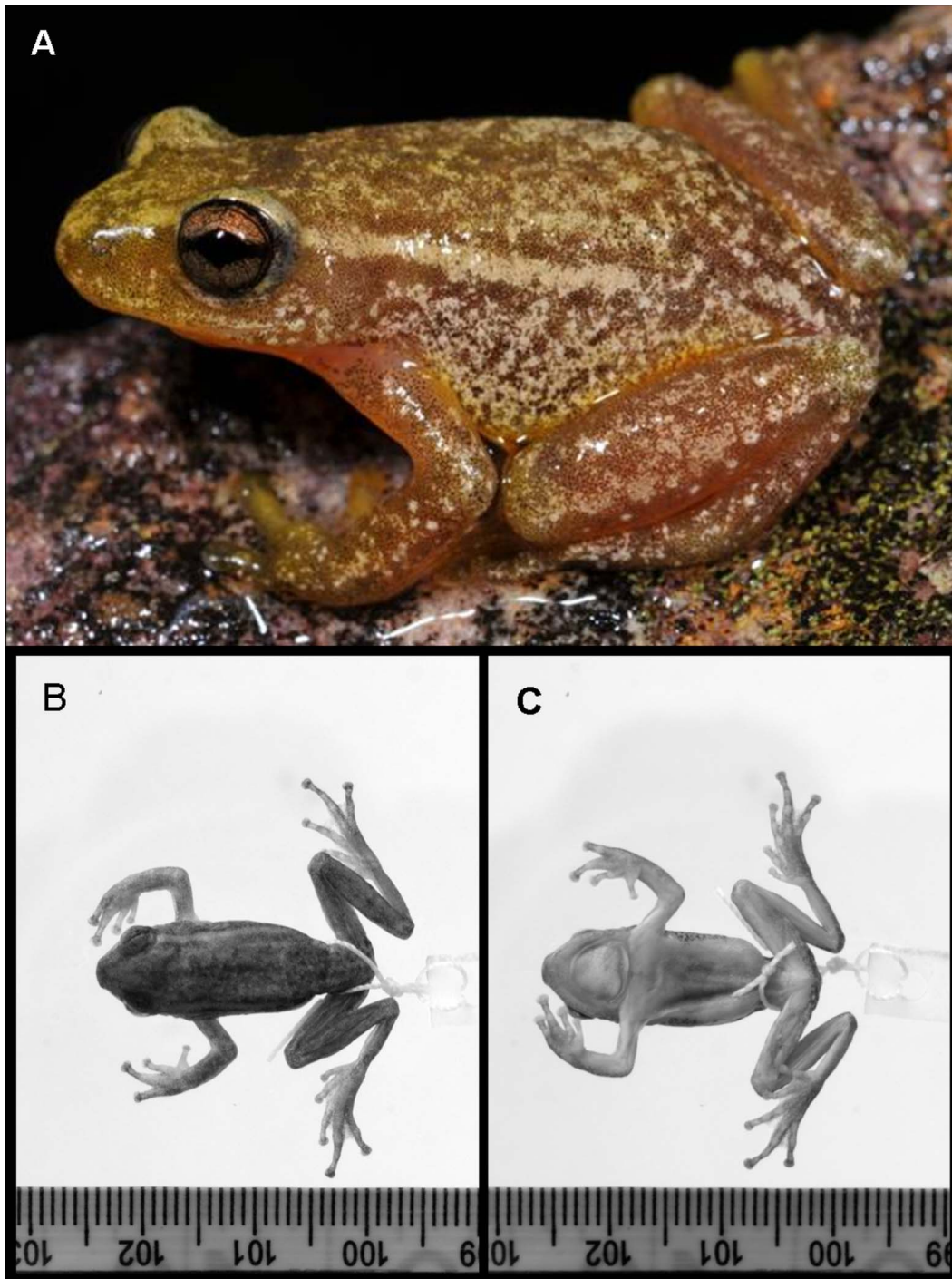


FIGURE 5. *Hyperolius chelaensis* **sp. nov.** male holotype (PEM A9223), A—lateral live, B—ventral preserve, C—dorsal preserve (Scale bar in mm).

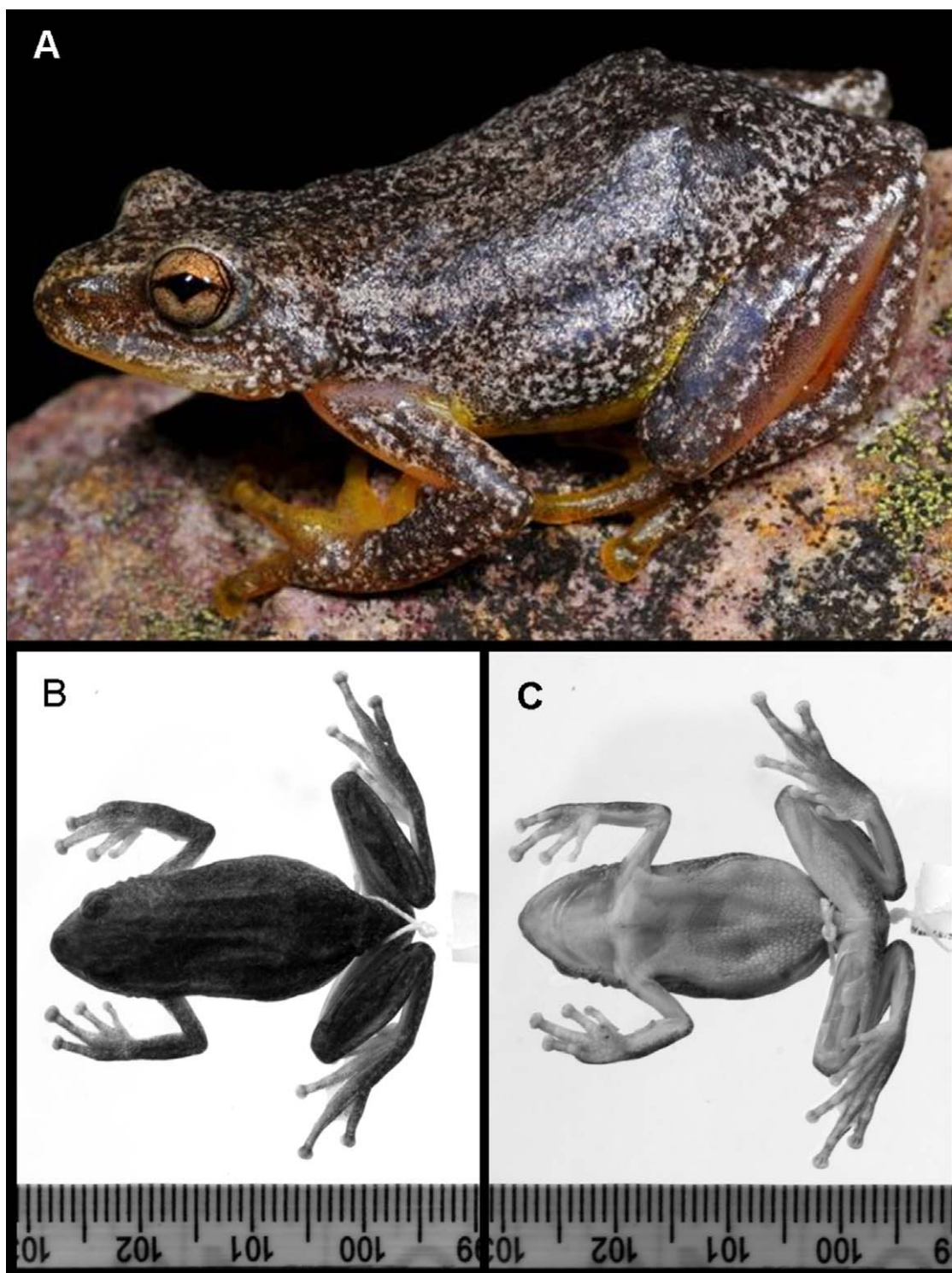


FIGURE 6. *Hyperolius chelaensis* sp. nov. female paratype (PEM A9224), A—lateral live, B—ventral preserve, C —dorsal preserve (Scale bar in mm).

Conservation. Additional surveys are needed in the Serra da Chela and adjacent mountains to determine the full distribution of the species, and thus assess its conservation status. At present it should be regarded as Data Deficient according to the IUCN criteria's (IUCN 2001). The current rate of habitat change occurring in Angola due to development pressures, and the lack of formally protected areas along the escarpment, are potential threats to this range-restricted species.

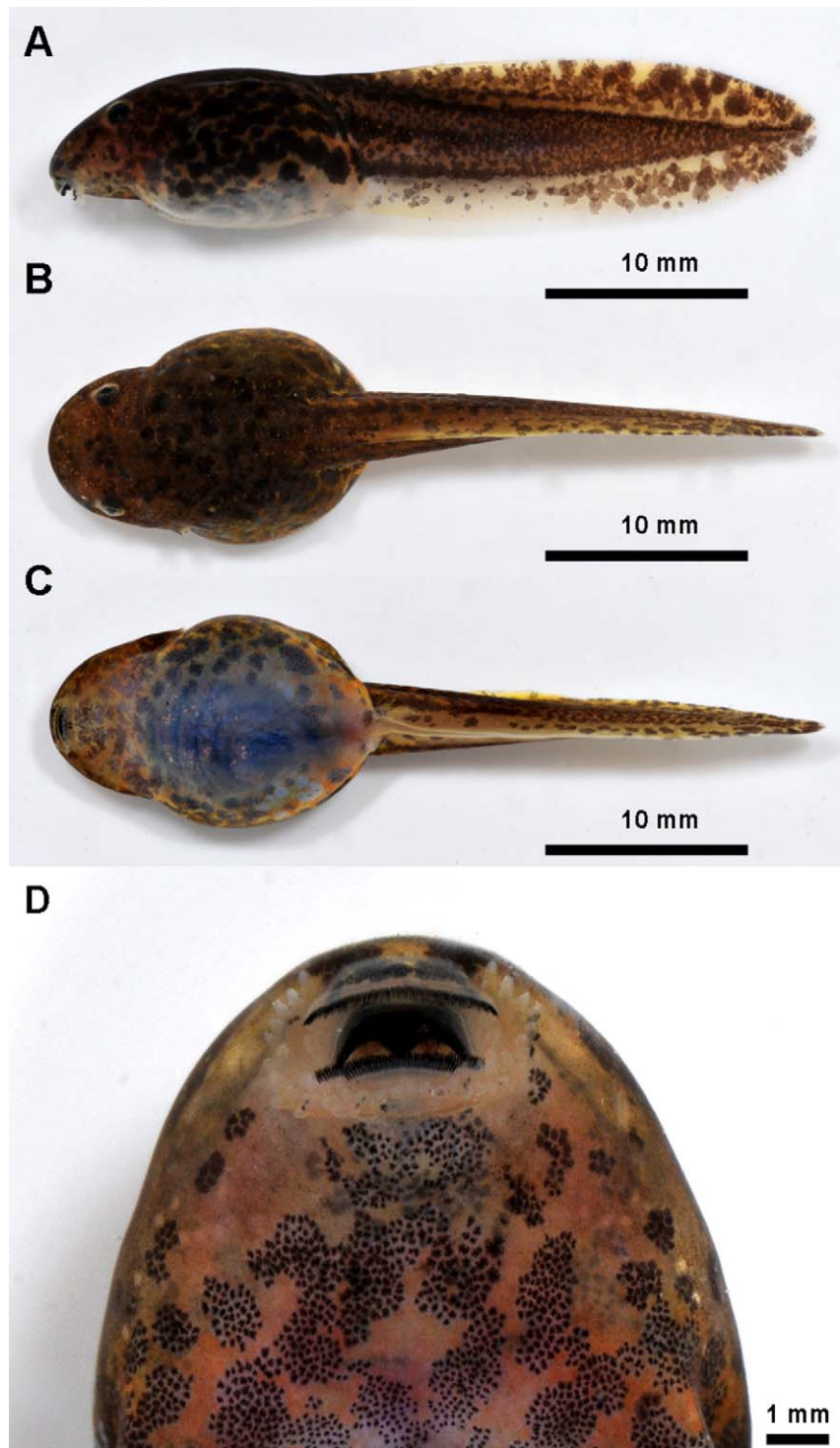


FIGURE 7. Tadpole of *Hyperolius chelaensis* sp. nov. (PEM T350) (A—lateral, B—dorsal, C—ventral and D—close-up of mouth).

Discussion

The phylogenetic analysis shows that the specimens from the Serra da Chela gorge form a well-supported monophyletic lineage of *Hyperolius*, which is sister to *H. cinereus* from Angola, and this clade in turn is sister to *H. platyceps*. Additional sampling and geographic coverage for this genus may reveal lineages not present in our phylogeny, and would assist to establish the evolutionary relationships among species. This, coupled with levels of

sequence divergence (uncorrected p-distances) that are comparable to that found in other species of *Hyperolius* (> 4 % pairwise difference—see Table 2), indicate that the individuals sampled from Serra da Chela gorge are a new taxon (see Results). Although sequence divergence values should not be the sole qualifier for species boundaries (Ferguson 2002), p-distances (>4%) are also comparable to that observed between other anuran species (e.g. Vences *et al.* 2005; Loader *et al.* 2006; Blackburn 2008; Turner & Channing 2008; Tolley *et al.* 2010a, 2010b) and approximately double that expected within a single species for this marker (Fouquet *et al.* 2007; Vieites *et al.* 2009; Schick *et al.* 2010).

While we have relied upon a single mitochondrial marker to guide this interpretation, this “barcoding” approach has proven reliable in amphibian studies for documenting undiscovered diversity (Vences *et al.* 2005, Vieites *et al.* 2009). Our rationalization is further supported by bioacoustics, colouration and habitat (see Results), providing multiple lines of evidence that support this new species (Vieites *et al.* 2009). The advertisement call of *Hyperolius chelaensis* **sp. nov.** differs from that of its closest relative (see Results). Overall, basic morphology and body proportions of the new species are similar to other *Hyperolius*, but due to the small number of adults collected (n=2) statistical analysis is not possible. The sister taxon, *H. cinereus*, is probably smaller in body size, with a longer snout (Table 3), and appears to be brightly coloured compared (Fig. 4A & B) to *Hyperolius chelaensis* **sp. nov.** In addition, the southern population of *H. cinereus* was sampled in a stream on montane plateau (Fig. 4C), a mere 5 km from the Serra da Chela gorge, yet sequence divergence between the two is in excess of 4%. The two habitats were markedly different (open montane grassland versus escarpment edge forest with deep shade, Fig. 4D–F), suggesting that further study may reveal additional ecological differences.

Remarkably, our sampled northern and southern populations that are referable to *H. cinereus* (Monard 1937, Laurent 1964) did not form a well supported monophyletic group, and sequence divergence between the two clades is also similar to that expected between species (>4%). The two populations do group together in each of the phylogenetic analyses, but this relationship was not statistically supported. Moreover, the sampling sites for the two populations are 1000 km apart and intervening material is lacking. It is therefore not possible to determine if isolation by distance is a factor, or whether these lineages may also constitute separate species. Further sampling in Angola is necessary to resolve this issue. Both Monard (1937) and Laurent (1964) inspected only preserved material and could only speculate on the colouration of *H. cinereus* in life, and provided no information on biology or habitat of the populations. We will re-describe *H. cinereus* based on additional live material, as well as discuss their taxonomic relationships, biology and habitat, elsewhere (Conradie *et al.* in prep.).

Acknowledgments

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Material examined

- Hyperolius cinereus* (southern population: PEM A9203, 9214, 9217 & 9219 (4 spec., 2 male & 2 female), stream under road before Estacao Zootecnica, near Humpata, Lubango, Huila Province, Angola (14°54'14.4" S; 13°19'32.0" E, 1413CD, 2089 m above sea level), collected by W. Conradie, W.R. Branch, J.G. Measey and K.A. Tolley, 16 January 2009; PEM A9202, 9204–9213, 9215, 9216, 9218, 9220–9222 (17 spec., 14 males & 3 females), waterfall below dams, on plateau above Estacao Zootecnica, near Humpata, Lubango, Huila Province, Angola (14°54'51.3" S; 13°18'49.9" E, 1413CD, 2144 m above sea level), collected by W. Conradie, W.R. Branch, J.G. Measey and K.A. Tolley, 14–15 January 2009.
- Hyperolius cinereus* (northern population: PEM A10040–10048, 10050–10056 & 10066 (17 spec., 16 males & 1 female), dambo before junction to base camp, Largo Carumbo, Lunda Norte Province, Angola (7°44'39.2" S; 19°57'16.8" E, 0719DB, 813 m above sea level), collected by W. Conradie, W.R. Branch, P. Vaz Pinto, S. Baptista & N. Baptista, 30 April and 2 May 2011; PEM A10057 (1 spec., 1 male), headwaters of Lovua, north of village Capaia, Lunda Norte Province, Angola (8°20'18.5" S; 20°14'33" E, 0820AC, 154 m above sea level), collected by W. Conradie, W.R. Branch, P. Vaz Pinto, S. Baptista & N. Baptista 5 May 2011.