

Genetic and acoustic characteristics of African Reed Frogs (genus *Hyperolius*) in the Kaffa area, south-western Ethiopia

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Abstract. The taxonomy and distribution of African Reed Frogs (genus *Hyperolius*) in sub-Saharan Africa remain subjects of ongoing debate. Currently, only four species have been reported from Ethiopia, and this genus remains one of the least studied in the country. The aim of this study was to characterise *Hyperolius* species in the Kaffa area of south-western Ethiopia using molecular and acoustic data. We conducted opportunistic and standardised visual encounter surveys, together with acoustic surveys. Species were identified using morphology and call characteristics and were validated through genetic barcoding based on mitochondrial *16S* rRNA gene sequences. Three *Hyperolius* species were identified in the study area: *Hyperolius kivuensis*, *H. howelli*, and *H. viridiflavus*. The uncorrected p-distances showed that intraspecific genetic distance was < 0.01 for each of the three species. Call analyses revealed significant differences among the three species in peak frequency and delta time. Despite growing interest in Ethiopian batrachofauna, more than 75 species in Ethiopia still lack formal call descriptions and genetic comparisons. This study helps fill this knowledge gap and improves our taxonomic understanding of Ethiopian anuran diversity.

Keywords. Anura, bioacoustics, call types, genetic distance, phylogeny

Introduction

Recent changes to the taxonomy of African frogs based on molecular and behavioural traits, together with broader geographic sampling, have led to the discovery of numerous new taxa (Channing et al., 2013, 2022; Bittencourt-Silva et al., 2017; Bwong et al., 2020). However, the lack of comprehensive phylogenetic analyses has made it difficult to evaluate the biogeography of particular amphibian groups (Husemann et al., 2014).

Studies of African Reed Frogs (Hyperoliidae: *Hyperolius*) remain limited, resulting in a complex and ambiguous taxonomy (Channing and Rödel, 2019). *Hyperolius* is the largest genus in the family, with about 145 recognised species (Frost, 2026 that

are distributed throughout Central, East, West, and southern Africa. The genus is taxonomically unstable (Schlötter, 1999; Channing et al., 2013, 2022) and shows extreme colour variation even within single populations (Wieczorek et al., 2000). As a result, species delimitation within the genus has proven challenging (Portik et al., 2019; Frost, 2026). However, many species can be identified using a combination of morphological traits, habitat, call, and distribution (Channing and Howell, 2006). DNA has been utilised in more recent evolutionary studies to identify cryptic genera (Channing et al., 2013; Bell et al., 2015; Loader et al., 2015) and to delimit species boundaries (Loader et al., 2015; Bell, 2016; Bwong et al., 2020; Lawson et al., 2023).

Comprehensive studies on Ethiopian frog biogeography have focused mainly on the genera *Leptopelis* (Mengistu et al., 2013; Reyes-Velasco et al., 2018; Tiutenko and Zinenko, 2019, 2021; Goutte et al., 2022) and *Ptychadena* (Mengistu et al., 2013; Freilich et al., 2014; Smith et al., 2017; Goutte et al., 2021), while other taxa have received less attention. Recent studies on selected *H. viridiflavus* populations have shown that some taxa currently treated as subspecies should be elevated to species status, and at least ten species have been identified (Wieczorek et al., 2000). These findings are based on phylogenetic analysis of the frogs' mitochondrial DNA (mtDNA) sequence

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data in combination with advertisement calls and the use of the phylogenetic species concept to determine species boundaries (Channing et al., 2013; Bell et al., 2015; Mindje et al., 2020). Such molecular data in the study of hyperoliid phylogeny not only supported the restructuring of taxonomy but have also contributed substantially to research on African amphibian biogeography (Wieczorek et al., 2000; Bell et al., 2015; Mindje et al., 2020).

In Ethiopia, the Kaffa area is known for its abundance of wetlands, riverine forests, and remnant primary forests, which provide favourable habitats for *Hyperolius* species. Largen and Spawls (2010) provided distribution data for the amphibians of Ethiopia. However, this information is now outdated and does not adequately reflect current distributions. They noted that four *Hyperolius* species occur in Ethiopia: *Hyperolius balfouri*, *H. kivuensis*, *H. nasutus*, and *H. viridiflavus*. On the other hand, Tiutenko et al. (2023) recently confirmed the presence of *H. howelli* in Harenna Forest, a highland natural forest in the Bale Mountains of Oromia, southeastern Ethiopia, whereas Gebresenbet et al. (2013) had previously identified frogs from this population as *H. nasutus*. Channing et al. (2013) had also suggested that *H. nasutus* is not present in Ethiopia and described its range as extending from northern Botswana through south-western Zambia to northern Angola (IUCN SSC, 2016).

Frog advertisement calls are widely recognised as species-specific (Köhler et al., 2017; Fan et al., 2019) and acoustic similarity is often strongly correlated with phylogenetic relatedness (Gingras et al., 2013). Therefore, advertisement calls are a relevant trait in taxonomy and biodiversity research and can help identify cryptic species in the field (Fan et al., 2019). The genus *Hyperolius* in particular includes several morphologically cryptic species that await species delimitation (Channing et al., 2013). However, descriptive information on the calls of many species in this genus remains scarce. Therefore, this study aimed to 1) describe the advertisement calls of the *Hyperolius* species found in the Kaffa area of south-western Ethiopia; 2) reconstruct a molecular phylogeny of selected *Hyperolius* species and discuss its biogeographic implications; 3) describe the current distribution of *Hyperolius* species in Kaffa.

Materials and Methods

Study area. The study was conducted in the localities of Alemgono, Bita, Gojeb, Komba, Sheda, and Shoreri

in the Kaffa area of south-western Ethiopia (Fig. 1). The Kaffa area is an important centre of biodiversity because of its rich fauna and flora (NABU, 2020). Furthermore, this area encompasses one of the last remaining forests in Ethiopia, which is of major importance for frog conservation. The area has a prolonged wet season (Getnet et al., 2023), which supports amphibian breeding.

Field surveys, specimen collection, and species identification. Fieldwork was conducted from July 2018 to February 2022, and covered both the wet season (June to September) and the dry season (December to February). The study area was stratified into three habitat types: riverine forest, wetland, and agricultural land (specifically enset plantations). Six study areas were selected, each including the three habitat types. A rectangular transect was established in each study area within each habitat type, resulting in 18 transects in total. Following Rödel and Ernst (2004), each transect consisted of a 600 m perimeter path comprising two 200-m segments running north-south and two 100-m segments running east-west, forming a 200 × 100 m rectangle. To ensure consistency during surveys, transect paths were kept clear, minimising disturbances to key habitat features. The starting point for each transect was consistently set at the southeast corner, maintaining a uniform geographic orientation across all sampling units (Rödel and Ernst, 2004). During surveys, all visible frogs within a one-meter radius of the transect were recorded. A combination

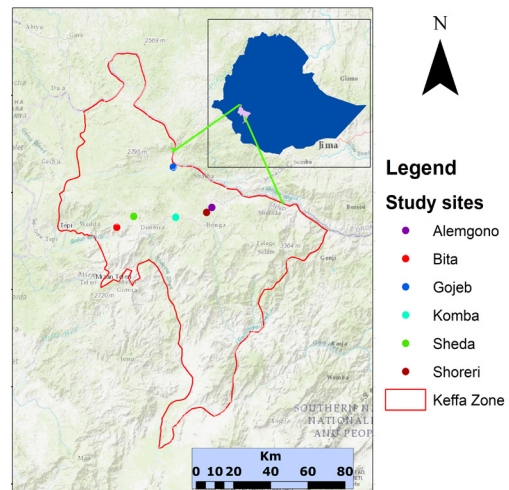


Figure 1. Survey localities for *Hyperolius* species in the Kaffa area, south-western Ethiopia.

of standardised visual encounter surveys and acoustic surveys was used to document the frog populations in each habitat (Heyer et al., 1994; Kok and Kalamandeen, 2008). Each transect was surveyed for six days: three during the wet season and three during the dry season. A team of three surveyed daily between 06:00–09:00 h and between 18:00–21:00 h, walking at a consistent pace and using a head torch during nocturnal sampling.

The taxonomic identity of individuals was determined using morphological characters from Largen and Spawls (2010), Spawls et al. (2023), and Channing and Howell (2006), and validated through genetic barcoding (see DNA extraction and analysis). The four *Hyperolius* species recorded from Ethiopia can be distinguished by body size, shape, colouration, and advertisement calls.

Hyperolius viridiflavus (Duméril & Bibron, 1841) is a robust, squat frog and the largest Ethiopian species, measuring up to 4.3 cm. Colouration is highly variable: females are often green or spotted, while males display whitish-grey to brown hues. A distinctive feature is the red colour of the toes and toe tips, with unexposed leg skin appearing pink. This species never exhibits broad dorsolateral or dorsal stripes, though lateral striping may occur (Spawls et al., 2023).

Hyperolius howelli Du Preez & Channing, 2013 is the smallest Ethiopian species, a tiny, slim frog reaching only about 2.5 cm. It has a sharply pointed snout and is predominantly green, with characteristic white dorsolateral stripes and fine dark speckling (Spawls et al., 2023; Tiutenko et al., 2023).

Hyperolius kivuensis Ahl, 1931 is moderately large, up to 3.8 cm, and can be recognised by a distinct dark eye stripe extending from the snout through the eye onto the flanks, bordered above by a pale line. Dorsal colouration is typically green or olive, and the toe tips are noticeably enlarged (Largen and Spawls, 2010).

Hyperolius balfouri (Werner, 1908) is similar in size to *H. kivuensis*, reaching up to 4.2 cm, and is quite variable in colour. It often displays a green dorsolateral stripe and may have a thin dark stripe through the eye, but lacks the distinct pale line above the eye stripe that characterises *H. kivuensis*. The underside is typically cream or yellow (Largen and Spawls, 2010).

Specimens were euthanised using 20% benzocaine or MS-222, and samples of muscle/liver tissue were excised and preserved in 97% ethanol in 2 ml Eppendorf tubes. Voucher specimens were first fixed in 97% ethanol and then preserved in 70% ethanol. All voucher specimens were deposited in the zoological museum of the Ethiopian Biodiversity institute, Addis

Ababa, Ethiopia.

DNA extraction and analysis. DNA extractions were carried out using the Qiagen DNeasy Tissue and Blood kit according to the manufacturer's instructions. Given the universal success of *16S* primers and the widespread use of this mitochondrial region for anuran barcoding, we sequenced at least two representatives from each morphologically determined species before deciding to amplify this region. The *16S* gene was amplified using the forward primer 5'-CGC CTG TTT AYC AAA AAC AT-3' and reverse primer 5'-CCG GTY TGA ACT CAG ATC AYG T-3' (Freilich et al., 2014). Polymerase chain reaction (PCR) amplification was conducted in a total reaction volume of 48 µL composed of 35.7 µL DEPC H₂O, 5 µL 10X Reaction Buffer, 4 µL MgCl₂, 1 µL dNTPs (10 µM), 1 µL of each primer (10 µM), and 0.3 µL NED Taq polymerase. We ran the PCR with an initial denaturation temperature of 94 °C for 2 minutes, followed by 35 cycles of 94 °C for 30 seconds, 48 °C for 30 seconds and 72 °C for 1 minute, finishing with an elongation step of 72 °C for 1 minute. All amplicons were sequenced by Beijing Genomics Institute (BGI) using Sanger sequencing in BGI Tech Solutions, Hong Kong Co., Limited, P.R. China.

The phylogram was inferred using Maximum Likelihood and the Tamura-Nei (Tamura and Nei, 1993) model of sequence evolution implemented in MEGA 11 (Tamura et al., 2021). Node support was evaluated using 1000 bootstraps and support values are indicated next to each node. Initial trees for the heuristic search were obtained by applying the Neighbour-Joining method to a matrix of pairwise distances estimated using the Tamura-Nei model (Tamura and Nei, 1993).

Advertisement call records. We recorded advertisement calls of the three *Hyperolius* species (*H. kivuensis*, *H. howelli*, *H. viridiflavus*) *in situ* (Sheda wetland, Komba wetland, and Gojeb wetland) at distances of 0.5–2.0 m to avoid near-field effects or substantial sound attenuation. The advertisement calls were recorded in June and July 2020 between 18:00–21:00 h, with air temperatures ranging from 17–25 °C and no wind, using a Sony Stereo Digital Voice Recorder. Most calls were recorded while males were perched on reed stems and leaves, along the banks of rivers, and in grassy wetlands.

Audacity 3.3.3 was used to process audio recordings collected in the study area. Calls were selected using the waveform window. For spectrogram and oscillogram images, call data were analysed using a Hann window ($n = 512$ samples) using Raven Pro 1.6.5 (Cornell Lab of

Ornithology, 2023). To balance temporal and frequency resolution, analyses were run with 50% overlap and a hop size of 256 samples. The frequency grid size was set at 93.8 Hz, and the Discrete Fourier Transform size was 512 samples (Lisa, 2023). We used the note-centred approach (identifying unbroken units of sound as notes and their entirety as a call) following Köhler et al. (2017). Wilcoxon rank-sum tests were used to compare call characteristics among the three *Hyperolius* species.

Results

Distribution of *Hyperolius* species. We recorded 999 individuals belonging to three species, *Hyperolius kivuensis*, *H. viridiflavus*, and *H. howelli*, in the Kaffa area (Fig. 2). These species were recorded in grassy wetlands and along the edges of riverine forest, at elevations between 1561–1857 m. *Hyperolius viridiflavus* and *H. kivuensis* were found in Komba, Sheda, Alemgono, and the wetlands around Bita Genet town (Table 1). *Hyperolius viridiflavus* was also recorded in riverine forest and ponds. A single *H. kivuensis* individual was observed in Shoreri wetland. *Hyperolius howelli* was recorded in the wetlands of

Gojeb, Alemgono, and Sheda. No *Hyperolius* species were observed in agricultural habitats. Across all species, more individuals were recorded during the wet season ($n = 573$) than during the dry season ($n = 426$).

Phylogenetic analysis. All observed individuals were identified morphologically and released immediately, except for specimens collected for molecular analyses: three *H. howelli* individuals, 12 *H. kivuensis*, and five *H. viridiflavus* (Appendix 1). Maximum likelihood phylogenetic reconstruction of the *Hyperolius* species recorded in the study area yielded a well-supported topology with a sum of branch lengths of 0.66 (Fig. 3). The phylogram revealed clear species-level separation among the three *Hyperolius* taxa examined, with bootstrap support values indicating robust phylogenetic relationships within this reed frog assemblage.

Uncorrected p-distances calculated in MEGA 11 showed that intraspecific genetic distance was < 0.01 for *H. kivuensis*, *H. viridiflavus*, and *H. howelli* (Fig. 3). *Hyperolius kivuensis* was genetically more divergent from *H. howelli* (16%) than to *H. balfouri* (8%).

Acoustic characteristics. Advertisement calls differed clearly among *Hyperolius kivuensis*, *H. howelli*,

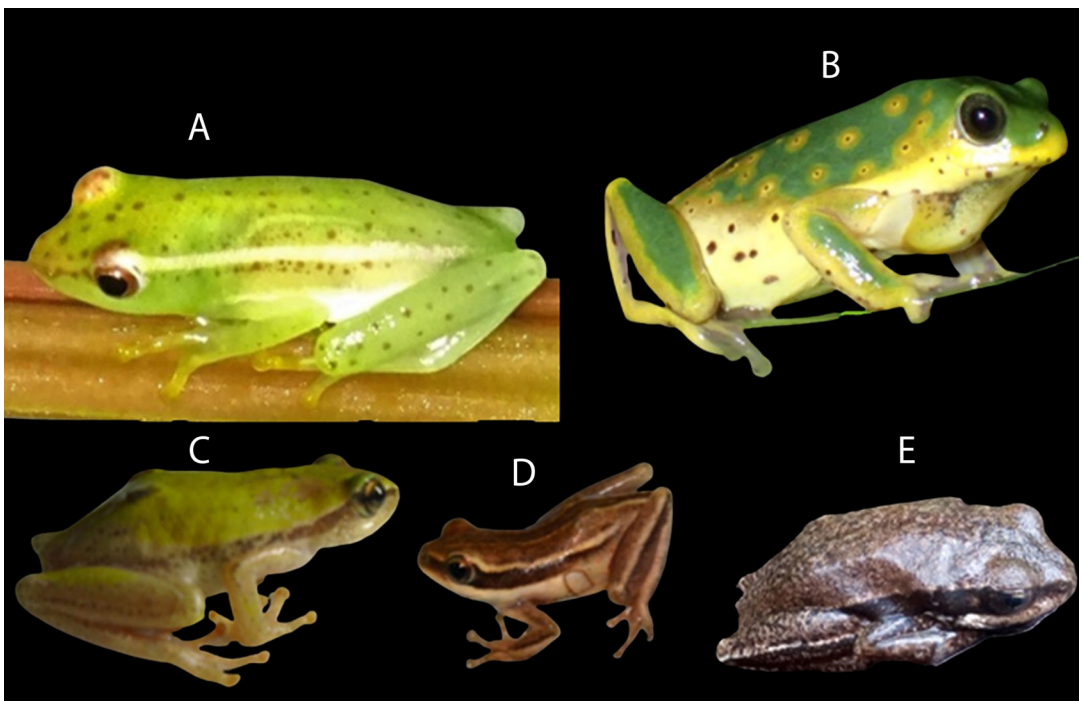


Figure 2. The three *Hyperolius* species recorded in the Kaffa area, south-western Ethiopia: (A) *Hyperolius howelli*, (B) *H. viridiflavus*, and (C–E) polychromatic variation in *H. kivuensis*. Photos by Abeje Kassie.

Table 1. The distribution and abundance of *Hyperolius* species in the Kaffa area, south-western Ethiopia.

Study site	Habitat	Abundance of <i>Hyperolius</i> species			Coordinates		
		<i>H. howelli</i>	<i>H. kivuensis</i>	<i>H. viridiflavus</i>	Latitude	Longitude	Elevation
Alemgono	Agricultural land	-	-	-	7.3548°N	36.2204°E	1739 m
	Riverine forest	-	-	-	7.3534°N	36.2365°E	1724 m
	Wetland	78	56	14	7.3625°N	36.2259°E	1711 m
Bita	Agricultural land	-	-	-	7.2702°N	35.7665°E	1928 m
	Riverine forest	-	-	-	7.2810°N	35.7914°E	1847 m
	Wetland	-	12	25	7.2702°N	35.7767°E	1853 m
Gojeb	Agricultural land	-	-	-	7.5640°N	36.0440°E	1583 m
	Riverine forest	-	-	-	7.5491°N	36.0525°E	1563 m
	Wetland	200	-	-	7.5503°N	36.0393°E	1561 m
Komba	Agricultural land	-	-	-	7.3138°N	36.0550°E	1868 m
	Riverine forest	-	-	13	7.3102°N	36.0677°E	1848 m
	Wetland	-	42	175	7.3165°N	36.0510°E	1824 m
Sheda	Agricultural land	-	-	-	7.2867°N	35.8339°E	1879 m
	Riverine forest	-	-	-	7.2795°N	35.8409°E	1797 m
	Wetland	200	150	33	7.3204°N	35.8483°E	1857 m
Shoreri	Agricultural land	-	-	-	7.3588°N	36.1874°E	1703 m
	Riverine forest	-	-	-	7.3626°N	36.2107°E	1671 m
	Wetland	-	1	-	7.3391°N	36.2001°E	1603 m

and *H. viridiflavus* (Table 2). Pairwise Wilcoxon rank-sum tests showed significant differences among all species pairs in both peak frequency and delta time (all $p < 0.001$). Bandwidth also differed significantly in all comparisons, except between *H. kivuensis* and *H. viridiflavus* ($p = 0.06$).

Oscillograms and spectrograms of the three species are shown in Figure 3. The call of male *H. kivuensis* is characterised by nine pulses over a 45-s recording (Fig. 4A). In *H. viridiflavus* and *H. howelli*, calls were continuous and repetitive and consisted of high-pitched notes (Fig. 4B, C).

Discussion

The Kaffa area is a highly biodiverse region characterised by habitats such as riverine forest and grassy wetlands (NABU, 2020). In this study, we recorded *Hyperolius kivuensis*, *H. viridiflavus*, and *H. howelli* in grassy wetlands and along riverine forest edges. This pattern is consistent with previous studies identifying wetlands as key habitats for *Hyperolius* species (Largen, 1998; Channing et al., 2002; Largen and Spawls, 2010; Mindje et al., 2020).

The occurrence of *H. viridiflavus* and *H. kivuensis* in several wetlands across the Kaffa area suggests that both species are relatively common and broadly distributed in the region. By contrast, *H. howelli* was recorded only in three wetlands, all associated with reed-grass

Table 2. Call duration, peak frequency and bandwidth of *H. kivuensis*, *H. howelli*, and *H. viridiflavus* recorded in the Kaffa area of south-western Ethiopia. Values indicate mean \pm standard deviation and values range between parentheses.

Species	Delta time (s)	No. pulses	Peak frequency (kHz)	Bandwidth (kHz)
<i>H. kivuensis</i>	0.56 \pm 0.25 (0.21–0.88)	9	2.5 \pm 0.06 (2.4–2.6)	2.8 \pm 0.13 (2.5–2.9)
<i>H. howelli</i>	0.13 \pm 0.07 (0.01–0.32)	Repeated	4.4 \pm 0.15 (3.8–4.7)	1.0 \pm 0.42 (0.52–3.7)
<i>H. viridiflavus</i>	0.07 \pm 0.02 (0.04–0.12)	39	2.8 \pm 0.03 (2.8–2.9)	2.6 \pm 0.84 (0.17–3.0)

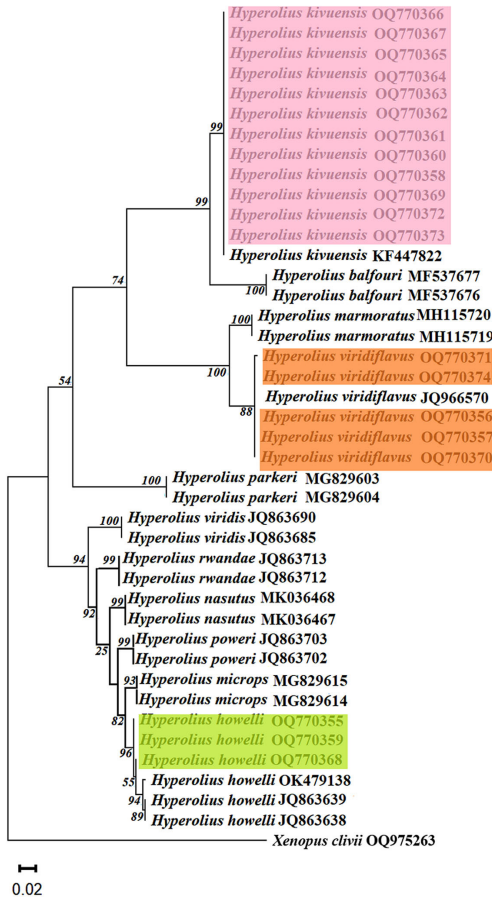


Figure 3. Maximum-likelihood phylogram of selected species of *Hyperolius*, rooted with *Xenopus clivii* as the outgroup. The analysis was based on 430 bp of the mitochondrial *16S* rRNA gene. Shaded taxa represent the species recorded in the Kaffa area, south-western Ethiopia.

habitat surrounded by dense forest, which may indicate narrower habitat requirements and a more restricted local distribution. The elevational range documented here (1561–1857 m) is consistent with previous records of *H. viridiflavus* and *H. kivuensis* in Ethiopia (Largen and Spawls, 2010).

Our analyses further indicate low intraspecific genetic divergence in all three species. Although frogs previously reported from Ethiopia as *H. nasutus* (Largen, 1998) were later referred to *H. acuticeps* by Channing et al. (2002), subsequent integrative work based on genetic distance, advertisement calls, and morphometrics recognised the Ethiopian populations as *H. howelli* (Channing et al., 2013). Within our

dataset, the relatively large genetic distance between *H. kivuensis* and *H. howelli* (16%) indicates that these two species are more distantly related to one another than either is to *H. viridiflavus*.

We found clear differences in male advertisement calls among the three *Hyperolius* species recorded in the Kaffa area. These differences involved both temporal and spectral traits, including pulse number, delta time, peak frequency, and bandwidth. *Hyperolius kivuensis* produced calls with nine distinct pulses, whereas *H. viridiflavus* produced calls with 39 pulses and *H. howelli* emitted a repetitive pulsed call. Taken together, these differences indicate marked acoustic divergence among the three taxa.

The strongest differences among species involved peak frequency and delta time, both of which separated all pairwise comparisons in our dataset. By contrast, bandwidth of *H. kivuensis* and *H. viridiflavus* did not differ significantly. The overall structure of the calls remained clearly distinct. In particular, the call of *H. viridiflavus* was readily recognisable as a short, loud note with a metallic, bell-like quality, making it easy to distinguish in the field from the calls of the other two species.

At a broader scale, these findings underline how limited our knowledge of Ethiopian *Hyperolius* diversity remains. According to Frost (2026, neighbouring East African countries such as Kenya, Tanzania, and Uganda support substantially higher numbers of *Hyperolius* species, whereas only four species are currently known from Ethiopia, three of which were recorded in the Kaffa area in this study. *Hyperolius balfouri*, although reported from Ethiopia, was not detected in Kaffa, which may indicate that its distribution in south-western Ethiopia is more restricted, possibly to lower elevations. Given the size and ecological diversity of the country, many potentially suitable areas remain insufficiently surveyed (Smith et al., 2017).

The recent description of numerous new frog species from Ethiopia (e.g., Goutte et al., 2019, 2021, 2022) further suggests that the country’s amphibian diversity is still underestimated. Additional integrative work combining field surveys, bioacoustics, and molecular data will likely reveal a more complete picture of *Hyperolius* diversity and distribution. In this context, the conservation of wetlands, riverine habitats, and other potential anuran breeding sites should be considered a priority, especially given the ongoing loss and degradation of amphibian habitats worldwide (Grant et al., 2016).

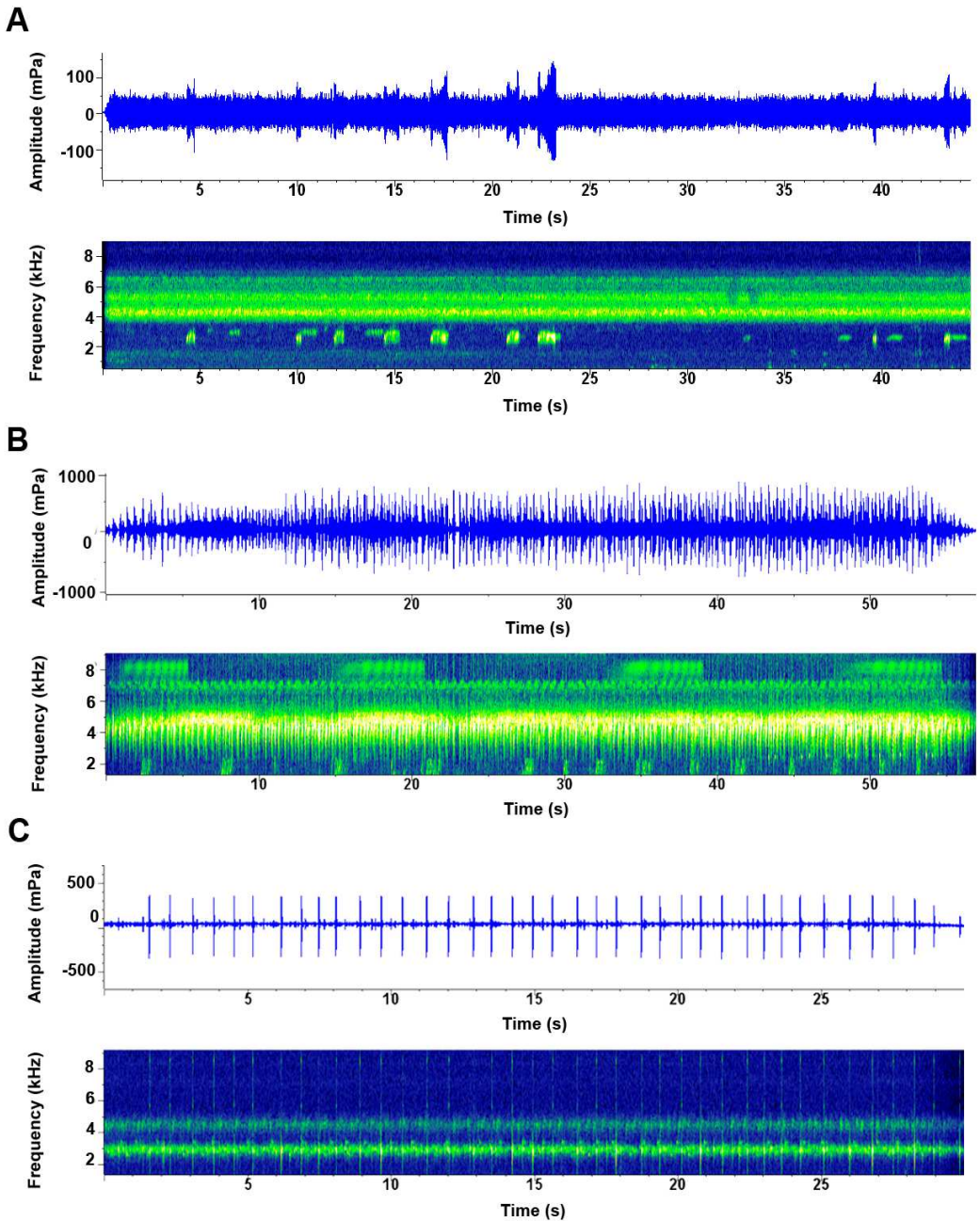


Figure 4. Oscillograms (top) and spectrograms (bottom) of advertisement calls of (A) *Hyperolius kivuensis*, (B) *H. howelli*, and (C) *H. viridiflavus* recorded in the Kaffa area, south-western Ethiopia.

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Appendix 1. List of *Hyperolius* specimen samples used for analysis, their sampling localities, voucher specimen numbers, and GenBank accession numbers.

Species	Museum voucher number	GenBank accession number	Locality	Source
<i>Hyperolius acuticeps</i>	-	JQ863691	Malawi	NCBI GenBank
<i>Hyperolius balfouri</i>	-	MF537676	South-eastern Gabon	NCBI GenBank
<i>Hyperolius balfouri</i>	-	MF537677	South-eastern Gabon	NCBI GenBank
<i>Hyperolius howelli</i>	AB3820	OQ770355	Kaffa, Ethiopia	This study
<i>Hyperolius howelli</i>	-	JQ863638	Madehani, Tanzania	NCBI GenBank
<i>Hyperolius howelli</i>	-	JQ863639	Madehani, Tanzania	NCBI GenBank
<i>Hyperolius howelli</i>	AB3824	OQ770359	Kaffa, Ethiopia	This study
<i>Hyperolius howelli</i>	AB3851	OQ770368	Kaffa, Ethiopia	This study
<i>Hyperolius howelli</i>	-	OK479138	Harrena, Ethiopia	NCBI GenBank
<i>Hyperolius kivuensis</i>	AB3822	OQ770358	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3828	OQ770360	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3829	OQ770361	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3836	OQ770362	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3837	OQ770363	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3838	OQ770364	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3839	OQ770365	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3840	OQ770366	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3841	OQ770367	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3852	OQ770369	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3956	OQ770372	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	AB3957	OQ770373	Kaffa, Ethiopia	This study
<i>Hyperolius kivuensis</i>	-	KF447822	Uganda	NCBI GenBank
<i>Hyperolius marmoratus</i>	-	MH115719	KwaZulu-Natal, South Africa	NCBI GenBank
<i>Hyperolius marmoratus</i>	-	MH115720	KwaZulu-Natal, South Africa	NCBI GenBank
<i>Hyperolius microps</i>	-	MG829614	Mozambique	NCBI GenBank
<i>Hyperolius microps</i>	-	MG829615	Mozambique	NCBI GenBank
<i>Hyperolius nasutus</i>	-	MK036467	Angola	NCBI GenBank
<i>Hyperolius nasutus</i>	-	MK036468	Angola	NCBI GenBank
<i>Hyperolius parkeri</i>	-	MG829603	Shimba Hills, Kenya	NCBI GenBank
<i>Hyperolius parkeri</i>	-	MG829604	Shimba Hills, Kenya	NCBI GenBank
<i>Hyperolius poweri</i>	-	JQ863702	Port Edward, South Africa	NCBI GenBank
<i>Hyperolius poweri</i>	-	JQ863703	Port Edward, South Africa	NCBI GenBank
<i>Hyperolius rwandae</i>	-	JQ863712	Mugesera wetland, Rwanda	NCBI GenBank
<i>Hyperolius rwandae</i>	-	JQ863713	Mugesera wetland, Rwanda	NCBI GenBank
<i>Hyperolius viridiflavus</i>	AB3817	OQ770356	Kaffa, Ethiopia	This study
<i>Hyperolius viridiflavus</i>	AB3819	OQ770357	Kaffa, Ethiopia	This study
<i>Hyperolius viridiflavus</i>	AB3835	OQ770370	Kaffa, Ethiopia	This study
<i>Hyperolius viridiflavus</i>	AB3963	OQ770371	Kaffa, Ethiopia	This study
<i>Hyperolius viridiflavus</i>	AB3812	OQ770374	Kaffa, Ethiopia	This study
<i>Hyperolius viridiflavus</i>	-	JQ966570	Rwanda	NCBI GenBank
<i>Hyperolius viridis</i>	-	JQ863685	Kaningina Forest Reserve, Malawi	NCBI GenBank
<i>Hyperolius viridis</i>	-	JQ863690	Kaningina Forest Reserve, Malawi	NCBI GenBank
<i>Xenopus clivii</i>	AB3816	OQ975263	Kaffa, Ethiopia	This study