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Museomics and integrative taxonomy reveal three new species of glandular viviparous tree toads (*Nectophrynoides*) in Tanzania's Eastern Arc Mountains (Anura: Bufonidae)

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Abstract

For the last century, herpetologists have referred to any *Nectophrynoides* Noble, 1926 toad characterized by a large, robust body, with large, distinct parotoid glands, as *Nectophrynoides viviparus* (Tornier, 1905). Consequently, *Nectophrynoides viviparus* is also considered to have the broadest distribution of all its congeners, with populations ranging from the Tanzanian Southern Highlands, close to the Tanzania-Malawi border, through the Udzungwa and Mahenge Mountains in the south to Uluguru, Rubeho, and Nguru Mountains in the central part of the Eastern Arc Mountains. However, there is underappreciated morphological diversity within what is generally referred to as *N. viviparus*, and various populations are isolated by large distances and geographical barriers. Recent molecular studies have shown that *N. viviparus* from the Southern Highlands, the type locality, is genetically distinct from all other *N. viviparus* populations in the Eastern Arc Mountains, suggesting the existence of a species complex warranting taxonomic revision. Here, we present an integrative taxonomic assessment of southern populations by supplementing the genetic results with the analysis of morphometric and morphological data for 257 specimens assigned to *N. viviparus*, including museomic data for name-bearing types. Based on the results, we describe three new species from the *N. viviparus* species complex, covering the southern Eastern Arc Mountains populations. Together with a revised morphological key to the genus and a gazetteer of known populations, we provide Extent of Occurrence and Area of Occupancy for *N. viviparus* sensu stricto and the new species to investigate their conservation status compared to other members of the genus.

Keywords

Archival DNA, bioacoustics, conservation, genetics, Mahenge Mountains, morphometrics, *Nectophrynoides viviparus*, *Nectophryne werthi*, Southern Highlands, species complex, Udzungwa Mountains

Introduction

Amphibians, especially in the tropics, often harbour cryptic morphological and genetic diversity, meaning that the true species richness of global biodiversity hotspots are likely underestimated (Carné and Vietes 2024; Ferreira et al. 2025). One hotspot of high species richness on the African continent is the Eastern Arc Mountain Range of Tanzania that consist of several ecologically isolated and fragmented mountains, where many amphibian species have evolved in allopatry, resulting in many cases of single-mountain endemism. These fragmented mountain and forest habitats of East Africa hold many species including charismatic vertebrate groups such as amphibians (Loader et al. 2010; Menegon et al. 2011b; Rovero et al. 2014; Lawson et al. 2023).

The genus *Nectophrynoides* Noble, 1926 belongs to the family Bufonidae and currently consists of 13 recognised species that are endemic to moist forests and grasslands in Tanzania. Members of *Nectophrynoides* exhibit viviparous reproduction, a trait that is extremely rare among anurans, with only 17 known viviparous species worldwide — of which 13 are species of *Nectophrynoides* (Liedtke et al. 2022). All species of *Nectophrynoides* are restricted to the Eastern Arc Mountains, except *Nectophrynoides viviparus* (Tornier, 1905), which is also found in the adjacent Southern Highlands of Tanzania (Barbour and Loveridge 1928; Loader et al. 2009; Menegon et al. 2011b). *Nectophrynoides viviparus* from the Southern Highlands in Tanzania was described by Gustav Tornier in 1905 under the name *Pseudophryne vivipara* and was later transferred to *Nectophrynoides* by Noble (1926). As currently understood, the species is easily distinguished from its congeners by its large and robust body characterised by distinct parotoid glands and enlarged glandular masses on the limbs. In subsequent years, *Nectophrynoides viviparus* was found more broadly in the Eastern Arc Mountains of Tanzania (Loader et al. 2009). However, Liedtke et al. (2016) showed that *N. viviparus* is a species complex; populations outside of the Southern Highlands assigned to this species are highly genetically distinct and could constitute several candidates for species-level recognition. This would match an overall pattern of high micro-endemism of amphibian populations in Afromontane regions such as the Eastern Arc Mountains and the Southern Highlands (Loader et al. 2009).

A key challenge in describing morphologically similar cryptic lineages is to correctly assign type material housed in museums, especially when series are old and have had confusing or complex curatorial histories. The description of *Nectophrynoides viviparus* by Tornier was based on a large type series of 33 specimens plus an unknown number of embryos or toadlets in the Museum für Naturkunde, Berlin, from the Ngozi Crater and Mount Rungwe in the Southern Highlands collected between 1899 and 1900 by Friedrich G. H. H. Fülleborn, as well as a series of specimens from "Daressalam" (Dar es Salaam) collected by Dr. Emil Werth. Assuming the type series was lost, Jean-Luc Perret proposed a neotype for *N. viviparus* (Perret 1972). This proposal was made in error as the designated neotype was not collected near the type localities (Loader et al. 2009). Eventually, the type series was re-discovered in the collection of the Museum für Naturkunde in Berlin, and a lectotype was designated from Fülleborn's numerous syntypes, setting the type locality as "Kratersee des Nyisilvulkans" (Crater Lake of the Nyisil Volcano, the Southern Highlands) (Loader et al. 2009). Nieden (1911) described *Nectophryne werthi* Nieden, 1911, based on part of the original *Nectophrynoides* syntype series of Tornier from "Daressalam", but the nomina were later synonymised by Perret (1972).

This study aims to assess the taxonomic distinctiveness of genetically divergent *Nectophrynoides* populations endemic to the Eastern Arc Mountains of Tanzania, in comparison to *N. viviparus* sensu stricto from the Southern Highlands of Tanzania. Specifically, we examine populations from the Mahenge and Udzungwa Mountains using an integrative approach that combines morphological, bioacoustic, and molecular data. We were further able to obtain mitochondrial DNA for name-bearing types of *N. viviparus* sensu stricto using a museomic approach, allowing us to unambiguously assign this name within the species complex to formally describe three new species of viviparous *Nectophrynoides* toads.

Materials and Methods

Morphology

Abbreviations of institutions are as follows: **AMNH** (prefix: A) (American Museum of Natural History, New York, USA), **BM**, **BMNH** and **NHM** (Natural History Museum, London, UK), **MHNG** (Museum d'Histoire Naturelle, Geneva, Switzerland), **MTSN** and **MUSE** (The Science Museum of Trento, Italy), **ZMB** and **MfN** (Zoologisches Museum, Humboldt Universität, Berlin, Germany and Museum für Naturkunde, Berlin, Germany), **ZMUC** (prefixes: R or H) and **NHMD** (Zoological Museum, University of Copenhagen, Denmark or Natural History Museum Denmark). Abbreviations of collectors are as follows: **JVL** (John V. Lyakurwa), **KMH** (Kim M. Howell field tags), **M** (Mette M. Westergaard field tags).

For clarity and ease of reference, we refer to the herein newly described species by their proposed names in the text, figures and tables. Formal diagnoses and descriptions are provided in the taxonomic section of the Results below.

A total of 257 specimens attributed to *Nectophrynoides viviparus* were examined and measured by the first author. All specimens are stored in 70% ethanol and housed in five different European museum collections, including ZMUC (114 specimens), NHM (49 specimens), ZMB (37 specimens), MHNG (29 specimens) and MUSE (28 specimens). A comprehensive list of field and museum catalogue numbers for examined specimens is provided in File S1.

Measurements were taken with a Mitutoyo electronic calliper (Product code: 500–158–30) accurate to 0.01 millimetres (mm) under a dissection microscope with strong lighting and automatically transcribed to a computer via a data transfer cable (Product code: 06AFM380C). The measurement scheme contains the following traits: Snout–urostyle length (**SUL**), snout–vent length (**SVL**), head length taken from the back of the jaw (corresponding to the posterior end of the angulosplenial) to tip of the snout (**HL**), head width at the back of the jaw (**HW**), nostril-nostril distance (**ND**), nostril-snout distance (**NSD**), eye–nostril distance (**END**), horizontal eye diameter (**ED**), horizontal tympanum diameter (**TYMP**), first fingertip width at the widest point (**F1W**), third fingertip width at the widest point (**F3W**), outer metacarpal tubercle length (**OMCL**), outer metacarpal tubercle width (**OMCW**), inner metacarpal tubercle length (**IMCL**), hand length taken from base of outer metacarpal tubercle to tip of third finger (**HAL**), forearm length taken from elbow to end of radioulna with arm held at a 90 degree angle (**FOL**), humerus length taken from elbow to axilla (**HUL**), first toe tip width at the widest point (**T1W**), fourth toe tip width at the widest point (**T4W**), outer metatarsal tubercle length (**OMTL**), inner metatarsal tubercle length (**IMTL**), foot length taken from the skin crease at the base of metatarsal tubercles to the tip of fourth toe tip (**FL**), metatarsus length (**ML**), tibia length taken from knee to metatarsus/tibia joint with leg folded (**TIL**), thigh length taken from knee to mid body line above the vent with the leg at a 90 degree angle outwards from the body (**THL**). Forelimb length (**FORL**) is HAL, FOL, HUL combined and hindlimb length (**HIL**) is FL, ML, TIL, THL combined. Illustrations of where measurements were taken are provided in File S2. These measurements were chosen based on previous publications with additional measurements added (Menegon et al. 2007; Loader et al. 2009). The measurements were taken on the right side of the specimen where possible.

Additional qualitative morphological traits were examined and noted. This included recording as distinct/indistinct or present/absent: parotoid glands, tympanum, tibial glands, white spot under eye, upper lip colour, supratympanic fold, dorsal pleat (mid-dorsal skin fold), canthus rostralis, tubercles on hands, and more detailed descriptions of skin texture, webbing on feet, femoral area, head shape, snout shape, finger and toe tip expansion, colour, pattern and markings (notes on morphological traits provided in File S1; illustrations of selected traits states used in the key are provided in File S2).

The colours used to describe colouration and patterning are white, ash grey (pale grey), cream (very pale yellowish brown), caramel brown (light brown), and tawny brown in three degrees: tawny brown (brown with a slight reddish tint); dark tawny brown (dark brown with a slight reddish tint); and very dark tawny brown (very dark brown, nearly black, with a slight reddish tint).

All quantitative linear measurements (after excluding SVL, which is redundant with SUL, and FORL and HIL as composites of limb measurements) were log10 transformed and corrected for allometry using SUL and the allometric growth equation implemented by the GroupStructure package (Onn and Grismer 2021) in R v. 3.2.1 (R Core Team 2015). To test for morphological shape differences in multidimensional space, first, an exploratory Principal Component Analysis (**PCA**) was performed followed by a Linear Discriminant Analysis (**LDA**) on the size-corrected data using the MASS package v7.3–60 (Venables and Ripley 2002), with a leave-one-out cross validation. A Multivariate Analysis of Variance (**MANOVA**) was also performed on the entire morphological matrix, followed by measurement-specific Analysis of Variance (**ANOVAs**) performed with the stats package (R Core Team 2015). For the ANOVAs, body size (SUL), head dimensions (HW and HL) and limb lengths (FORL and HIL) were compared, as representative measurements of body proportions. Specimens were not sexed because external sexual differences in *Nectophrynoides* are subtle, and reliable determination of sex would require dissection; therefore, females and males were not analysed separately. Juveniles (an approximate cut-off of SUL <17 mm was used based on follicular development of investigated individuals and presence of adult characteristics) were excluded from the analysis. Data, R code and additional analyses and plots for the linear morphometric analyses are deposited online (https://doi.org/10.5281/zenodo.17277236).

Bioacoustics

There are two available, and a number of unpublished, recordings of male calls attributed to the *Nectophrynoides viviparus* complex: One call recorded 26th of January 2011 in Mdandu Forest Reserve (**F.R.**) in the Southern Highlands and two calls recorded in January 1999 in Mkaja near Kihanga in Uzungwa Scarp Nature Forest Reserve (**N.F.R.**) in the southern Udzungwa Mountains in the Eastern Arc Mountains, all recorded by Michele Menegon. The two available recordings are deposited online (https://doi.org/10.5281/zenodo.17277236). Recording from the Southern Highlands was obtained using an Olympus LS-10 PCM digital stereo audio recorder equipped with a Sony directional microphone, and recordings in the Udzungwa Mountains were obtained using a Sharp MT 877 minidisc recorder with a Sony TCM directional microphone. The recordings were digitized and resampled at 44.1 kHz and 32-bit float resolution using the software Audacity v. 3.6.4 (Audacity Team 2024) and analysed in R-studio v. 4.3.2 (Posit Team 2025) using the FSA (Ogle et al. 2023) and ggplot2 (Wickham 2016) packages.

The following variables were measured and analysed: call duration, call interval, dominant frequency, number of pulses per call and pulse duration (Köhler et al. 2017). Pulse duration was measured from the start of one pulse to the start of the next pulse. We compared these variables between *N. viviparus* sensu stricto from the Southern Highlands, and *N. uhehe* **sp. nov.** from the Udzungwa Mountains using non-parametric tests, as data was not normally distributed (Shapiro-Wilk test, p-value <0.05).

To test overall group differences in our dataset, we applied a Mann-Whitney U test (Wilcoxon rank-sum test) followed by the Bonferroni correction for adjusted p-values.

Museomics

Name-bearing types of *Nectophrynoides viviparus* sensu stricto (ZMB 21775, 25261, 25312, 71187, 71193), deposited in the wet collection of the Museum für Naturkunde in Berlin, were sampled using minimally invasive methods targeting either muscle tissue from the thigh or liver tissue. Sampling was done with heat and UV sterilised scissors and tweezers, and the sample was stored in a tube with pure ethanol that had been previously filled in a laboratory naïve to molecular work with the focal group. Samples were processed at the University of Potsdam (Germany) in laboratories dedicated to the work with historical samples and fully compliant with all necessary standards (Fulton and Shapiro 2019). Prior to DNA extraction, the samples were weighed and incubated in a Guanidine Thiocyanate (**GuSCN**) based extraction buffer solution at 37°C overnight. A total volume of 25 µl genomic DNA was extracted the following day according to the protocol of Rohland et al. (2004), following several consecutive steps as outlined in Straube et al. (2021). DNA yield for each sample was then quantified based on 1 µl DNA extract using the Qubit dsDNA HS Assay Kit 0.2–100 ng/µl (Life Technologies, Carlsbad, California, US) according to the instructions of the manufacturer. Subsequently, 13 ng of DNA per sample were used as an input for a single-stranded library preparation following the protocol of Gansauge et al. (2017). Extraction and library blanks were included throughout the whole procedure to monitor for potential contamination. Final library concentrations and fragment length distributions were evaluated using a 2200 TapeStation assay (Agilent Technologies, Santa Clara, California, US). Sequence data for individual specimens were then obtained via shotgun sequencing, generating approximately five million 75-bp single-end reads on an in-house Illumina NextSeq 500/550 platform at the University of Potsdam, following the protocol described in Paijmans et al. (2017). The obtained reads were processed on the High-Performance-Computing (**HPC**) Cluster of the University of Potsdam. Read quality was assessed twice using FastQC (https://www.bioinformatics.babraham.ac.uk), both before and after trimming Illumina adapter sequences and removing reads shorter than 30 bp with cutadapt v1.12 (Martin 2011). Subsequent mapping of filtered reads against 12S and 16S ribosomal RNA (**rRNA**) mitochondrial DNA (**12S** and **16S**) was performed in Geneious Prime v. 2025.0.3 (Biomatters Ltd., Auckland, New Zealand) using a sequence of *Nectophrynoides tornieri* available on GenBank (16S: PQ280311; 12S: DQ283413) as a reference. Individual mapping was run for 100 iterations using a mapping quality filter of 30 and a mismatch threshold of 15%. The obtained contigs were visually checked to assure consistency and assembled into a consensus sequence with missing sections in-between contigs coded by the letter "N". Notes on sampling and the quality of sequences obtained from museomics are provided in File S3.

To assign type specimen sequences to phylogenetic lineages, the newly generated fragments of the mitochondrial marker 12S, 16S and cytochrome c oxidase subunit I (**COI**) sequences were aligned to those of the *Nectophrynoides viviparus* lineages presented in Liedtke et al. (2016). The full list of new sequences is provided in File S4. Sequences were aligned with the MAFFT v. 1.5.0 (Katoh et al. 2002) plug-in using the E-INS-i setting in Geneious Prime v. 2023.2.1 (Biomatters Ltd., Auckland, New Zealand). The position of fragments of the type sequences had to be adjusted manually to improve their alignment with the rest of the sequences. We then used IQ-TREE v. 2.2.0 (Minh et al. 2020) to reconstruct the phylogeny of the concatenated alignment (1971 bp in length), using the MFP+MERGE model with 5000 bootstrap iterations (Kalyaanamoorthy et al. 2017). This model finds the best partitioning scheme for 12S, 16S and each codon position of COI as a potentially independent partition. The outgroup was set as *Churamiti maridadi* Channing & Stanley, 2002, based on previous phylogenetic analysis identifying it as the sister taxon to *Nectophrynoides* (Liedtke et al. 2017; 2024). The proportions of uncorrected pairwise sequence differences in the 16S alignment were calculated using the R package 'ape' v5.7–1 (Paradis and Schliep 2019), ignoring sites where at least one of the pairs had missing data, and excluding all museomics sequences, which were, at times, highly fragmented. All newly generated sequences were submitted to GenBank (see File S4 for accession numbers).

Taxonomic methodology

The taxonomic approach for the description of three new species is based on multiple lines of evidence (Padial et al. 2010) to distinguish species, including a >3% pairwise difference in mitochondrial 16S rRNA fragments as threshold for species distinction as applied in Vieites et al. 2009, differences in external morphology (based on linear measurements and discrete characters) and, if available, bioacoustics differentiation in male calls. We follow the general lineage / unified species concept (de Queiroz 1998, 1999, 2005, 2007), treating species as lineages on a distinct evolutionary trajectory, and using our integrative taxonomic approach as evidence of supporting species criteria, such as morphological and genetic isolation.

Results

Phylogenetics

The sequence fragments obtained from the type series of *Nectophrynoides viviparus* correspond to two distinct phylogenetic lineages identified by Liedtke et al. (2016)

(Fig. 1). The type specimens ZMB 21775, 25312, 71193, 25261 and 71187, originally referred to as *Pseudophryne vivipara*, cluster with strong support (100% bootstrap support [BS]) with individuals from the Southern Highlands. The paralectotype specimens from "Daressalam" ZMB 21784, 25296, 21788 and 25297 (also part of Nieden's type series of *Nectophryne werthi* (Nieden 1911; Loader et al. 2009) cluster with moderate support (81% BS) with more recent specimens collected from the Uluguru Mountains. Thus, *Nectophrynoides* cf. *viviparus* ("*werthi*") can therefore be resurrected from synonymy (where it was placed by Perret [1972]) for the lineage from the Uluguru Mountains, as these specimens are indeed distinct from the lineage that now contains the lectotype of *N. viviparus* (Loader et al. 2009). However, this species is outside the geographic scope of this study and will be re-described in a forthcoming study (Lyakurwa et al. in prep).

Three further lineages corresponding to the *Nectophrynoides viviparus* morphotype were highlighted by Liedtke et al. (2016) including two from the Udzungwa Mountains and a third from the nearby Mahenge Mountains (Fig. 2). These represent lineages that are genetically distinct from *N. viviparus* sensu stricto and are herein described as new species. *Nectophrynoides saliensis* **sp. nov.** from Mahenge is sister to *N. viviparus* sensu stricto (3.110–3.349% uncorrected 16S pairwise distance). This clade is moderately well-supported (73% BS). *Nec-*

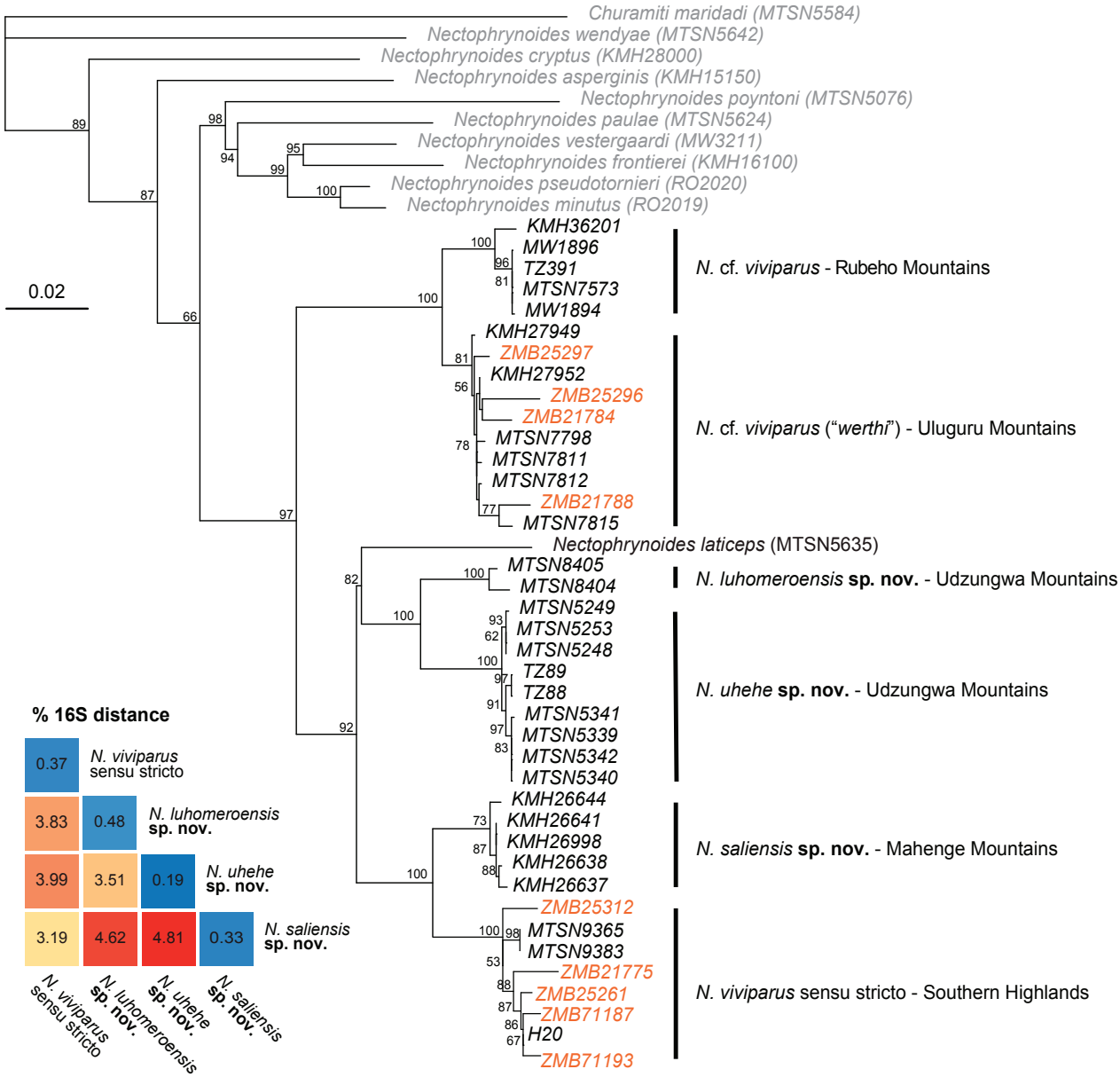

Figure 1. Maximum likelihood phylogeny of glandular tree toads inferred from the three mitochondrial markers (*Nectophrynoides viviparus* complex). ModelFinder recovered three partitions of the data (Partition 1: 12S,16S - TPM2u, Partition 2: COI_p1, COI_p2 - HKY, Partition 3: COI_p3 - TIM2). Branch supports refer to bootstrap values, and branch lengths represent the number of nucleotide substitutions per site (see scale bar). *Nectophrynoides* species not part of the *N. viviparus* complex are shown in grey, and type material for which sequence information was obtained via museomics are shown in orange. Heatmap inset shows mean uncorrected 16S genetic pairwise distances.

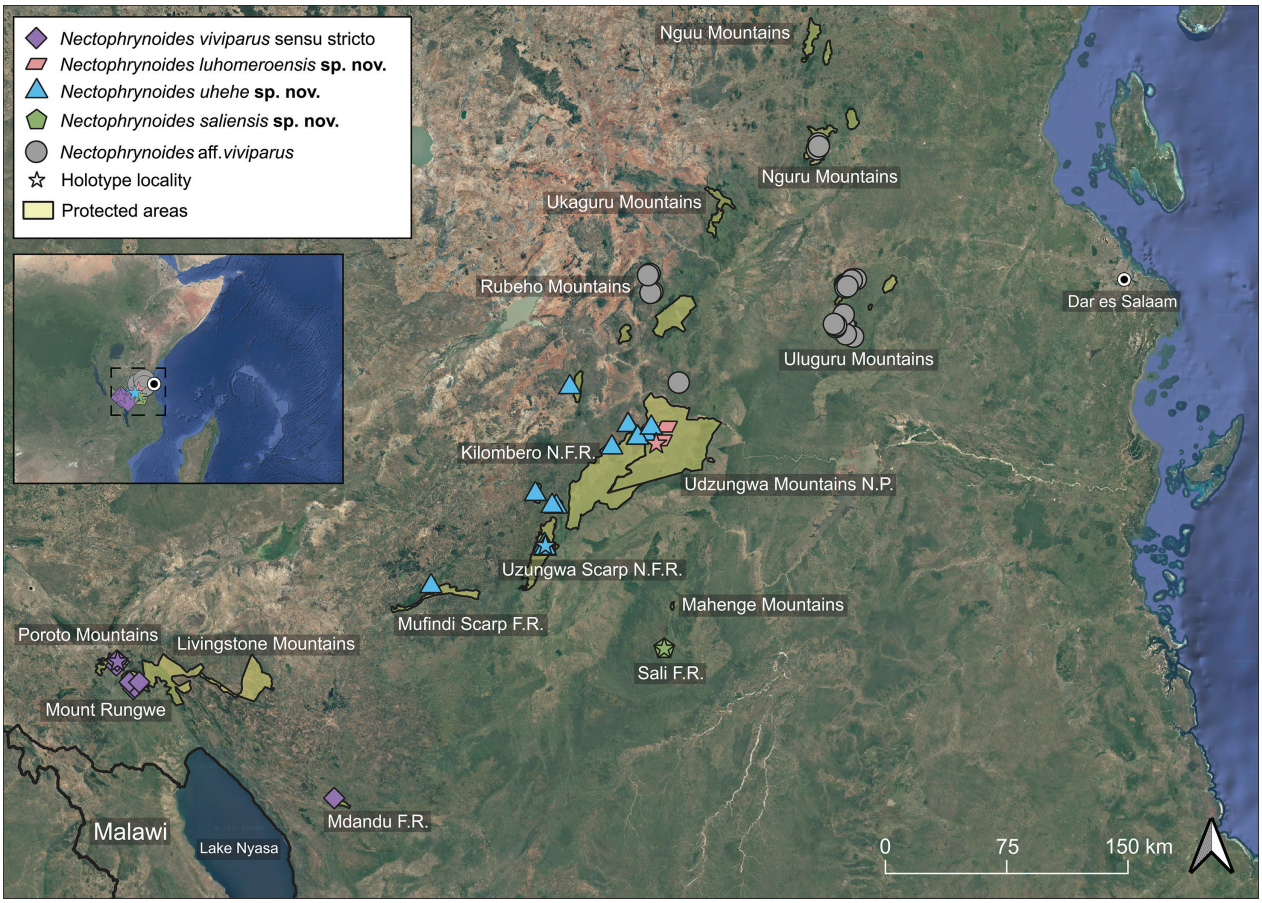

Figure 2. Distribution of the *Nectophrynoides viviparus* complex in the Eastern Arc Mountains and the Southern Highlands in Tanzania. Abbreviations: Forest Reserve (F.R.); Nature Forest Reserve (N.F.R.); National Park (N.P.). Basemap: Google Maps (map data © 2025 Google). Map created in QGIS v. 3.38.3 (QGIS.org 2025). Scale bar: 150 km.

tophrynoides luhomeroensis **sp. nov.** and *N. uhehe* **sp. nov.** (3.125–4.077% uncorrected 16S pairwise distance between them), both from the Udzungwa Mountains form a well-supported clade (100% BS), more distantly related to *N. viviparus* sensu stricto (3.589–4.067% and 3.846–4.317% uncorrected 16S pairwise distance, respectively; Figure 1 and Table 3). Furthermore, *N. laticeps* forms the sister species to the Udzungwa Mountains species, but this is only moderately supported by the data (82% BS).

Liedtke et al. (2016) already showed that *Nectophrynoides laticeps* falls within the *N. viviparus* species complex, even though the species is morphologically highly distinct from any known member of that group (Channing et al. 2005). Our results also recover this relationship, and like Liedtke et al. (2016), we found that the species is sister to what they referred to as "*Nectophrynoides* sp04" and "*Nectophrynoides* sp06", which we here describe as *N. luhomeroensis* **sp. nov.** and *N. uhehe* **sp. nov.**, respectively.

Morphometrics

A total of 257 adult specimens (8 *Nectophrynoides luhomeroensis* **sp. nov.**, 6 *N. saliensis* **sp. nov.**, 195 *N. uhehe* **sp. nov.**, 48 *N. viviparus* sensu stricto) were included in the morphological analysis, with minimum, maximum, and mean values with standard deviations for all measurements provided in Table 1; raw data are provided as File S1.

The PCA and LDA were largely congruent, showing overlaps in morphospace between all species except for *N. saliensis* **sp. nov.** when plotting the first two component axes (Fig. 3). The LDA on size-corrected measurements was able to classify species with 90.1% accuracy, with *N. saliensis* **sp. nov.** being the most distinct of the four species (primarily driven by head shape; HW and HL), with the remaining three being largely overlapping in morphology (Fig. 3A). A MANOVA on size corrected measurements also recovered a significant difference in multivariate morphospace between species ($df = 3.248$, Pillai = 1.257, $p<0.001$) with subsequent ANOVAs on representative measurements (see Materials and Methods) finding differences in body size ($F = 7.697$, $df = 3.248$, $p<0.001$), allometry-corrected head width (HW; $F = 11.070$, $df = 3.248$, $p = 0.008$), allometry-corrected head length (HL; $F = 7.478$, $df = 3.248$, $p = 0.013$), allometry-corrected forelimb length ($F = 47.43$, $df = 3.248$, $p<0.001$) and allometry-corrected hindlimb lengths ($F = 17.230$, $df = 3.248$, $p<0.001$). Significance of pairwise differences based on post hoc Tukey tests are shown in Fig. 3B. Compared to *N. viviparus* sensu stricto, only *N. uhehe* **sp. nov.** is significantly larger, with slightly larger head and limb proportions. *Nectophrynoides luhomeroen-*

Table 1. Minimum (**Min**), maximum (**Max**), mean and standard deviation (**SD**) for each measured character in mm for species of *Nectophrynoides viviparus* sensu stricto, *N. uhehe* **sp. nov.**, *N. luhomeroensis* **sp. nov.**, *N. saliensis* **sp. nov.** For measurement abbreviations, see Materials and Methods. For full raw data, see File S1.

	***N. viviparus* sensu stricto**		***N. luhomeroensis* sp. nov.**		***N. uhehe* sp. nov.**		***N. saliensis* sp. nov.**	
	Mean+SD	**Min-Max**	**Mean+SD**	**Min-Max**	**Mean+SD**	**Min-Max**	**Mean+SD**	**Min-Max**
SUL	24.76±4.75	18.84–37.24	21.79±3.77	18.40–30.01	29.83±8.61	17.22–52.45	26.30±5.02	20.78–34.31
HL	9.03±1.64	7.02–13.38	7.83±1.36	6.60–10.9	10.85±2.80	6.74–17.66	10.21±1.75	8.38–13.12
HW	9.34±1.73	7.2–14.39	8.13±1.22	6.92–10.70	11.37±3.36	6.18–20.10	10.74±1.88	8.64–13.58
ND	1.96±0.43	1.40–3.00	1.59±0.47	1.30–2.74	2.51±0.70	1.42–4.34	1.87±0.35	1.45–2.34
NSD	1.15±0.20	0.83–1.65	1.07±0.29	0.91–1.78	1.46±0.38	0.80–3.01	1.17±0.21	0.93–1.47
END	2.07±0.37	1.51–2.90	1.75±0.17	1.63–2.14	2.36±0.60	1.34–4.11	2.39±0.38	2.02–3.01
ED	2.93±0.42	2.39–4.43	2.70±0.36	2.20–3.38	3.58±0.90	2.04–6.10	3.09±0.42	2.60–3.64
TYMP	0.99±0.23	0.70–1.78	0.85±0.17	0.59–1.09	1.22±0.40	0.51–2.33	0.98±0.24	0.76–1.38
F1W	0.54±0.14	0.35–0.98	0.51±0.15	0.41–0.88	0.70±0.24	0.32–1.35	0.45±0.09	0.36–0.62
F3W	0.59±0.14	0.41–0.98	0.60±0.13	0.50–0.91	0.80±0.26	0.38–1.54	0.50±0.14	0.38–0.76
OMCL	1.21±0.31	0.84–2.16	1.13±0.32	0.86–1.85	1.62±0.55	0.66–3.33	1.10±0.30	0.65–1.50
OMCW	1.12±0.26	0.77–1.96	1.02±0.22	0.81–1.48	1.40±0.50	0.54–3.07	0.95±0.15	0.74–1.14
IMCL	0.83±0.24	0.54–1.61	0.79±0.18	0.54–1.04	1.18±0.43	0.46–2.47	0.72±0.13	0.53–0.86
HAL	7.05±1.62	4.98–11.93	6.37±1.32	5.36–9.43	9.24±3.00	4.80–16.66	6.87±1.45	5.06–8.74
FOL	5.73±1.37	4.03–8.92	4.99±1.26	4.02–7.96	7.72±2.39	4.25–13.85	6.42±1.20	5.00–8.21
HUL	4.38±1.14	3.25–7.24	4.46±0.66	3.70–5.89	6.11±1.68	3.28–11.47	5.06±0.90	4.13–6.38
T1W	0.52±0.13	0.35–0.88	0.52±0.13	0.44–0.83	0.75±0.27	0.35–1.54	0.48±0.15	0.31–0.74
T4W	0.58±0.12	0.43–0.91	0.58±0.14	0.47–0.91	0.82±0.28	0.40–1.78	0.50±0.11	0.37–0.69
OMTL	0.95±0.24	0.63–1.62	0.88±0.25	0.64–1.42	1.32±0.50	0.56–2.67	0.84±0.16	0.65–1.04
IMTL	1.33±0.35	0.90–2.23	1.23±0.39	0.92–2.09	1.77±0.64	0.69–3.33	1.05±0.15	0.87–1.26
FL	10.64±2.55	7.53–17.29	9.57±1.96	7.75–13.95	14.48±4.95	6.71–26.29	10.98±2.65	8.07–14.96
ML	6.19±1.45	4.67–10.63	5.30±1.17	4.26–8.04	8.08±2.51	3.87–14.01	6.44±1.28	5.02–8.10
TIL	10.23±2.44	7.31–16.87	8.67±1.59	7.33–12.34	12.64±3.80	7.03–22.21	11.32±2.29	8.84–14.74
THL	10.13±2.37	7.36–16.26	8.55±1.76	7.28–12.69	12.55±3.74	7.07–22.50	10.96±2.28	8.35–14.35
FORL	17.16±4.04	12.44–26.94	15.82±3.20	13.27–23.28	23.07±6.95	12.70–40.04	18.34±3.39	14.19–23.33
HIL	37.19±8.75	26.87–60.92	32.08±6.45	27.00–47.02	47.76±14.91	25.77–84.71	39.70±8.46	30.50–52.15

Table 2. Comparison of acoustic variables between *Nectophrynoides viviparus* sensu stricto and *N. uhehe* **sp. nov.**, values are visualised by means ± standard deviations. P-values are from Mann-Whitney U test (Wilcoxon rank-sum test) and were adjusted for multiple testing using the Bonferroni correction.

Variable	***N. viviparus* sensu stricto (mean ± SD)**	***N. uhehe* sp. nov. (mean ± SD)**	**W-value**	**P-value**	**P-value (corrected)**
Dominant frequency (Hz)	1877.71 ± 54.62	1891.64 ± 7.17	100	0.251	1.00
Call duration (s)	0.23 ± 0.03	0.28 ± 0.03	**139**	**0.0017**	**0.0086**
Call interval (s)	16.00 ± 5.29	3.24 ± 1.27	**0**	**< 0.001**	**0.0015**
Pulse number	13.43 ± 1.27	25.27 ± 3.66	**154**	**< 0.001**	**0.004**
Pulse duration (s)	0.017 ± 0.001	0.011 ± 0.001	**0**	**< 0.001**	**0.005**

sis **sp. nov.** and *N. saliensis* **sp. nov.** are both similar in size to *N. viviparus* sensu stricto, but *N. saliensis* has a distinctively larger head relative to body size than all other species. For more comprehensive differences, see diagnoses of the respective new species below.

Bioacoustics

Statistical comparison of call parameters (see Table 2) reveals that the two species differ significantly in call duration, call interval, pulse number and pulse duration, but not in dominant frequency. Thus, bioacoustics supports the recognition of these two lineages as separate species; calls are needed from other species-level lineages to understand the ability of bioacoustics to discriminate all species in this complex.

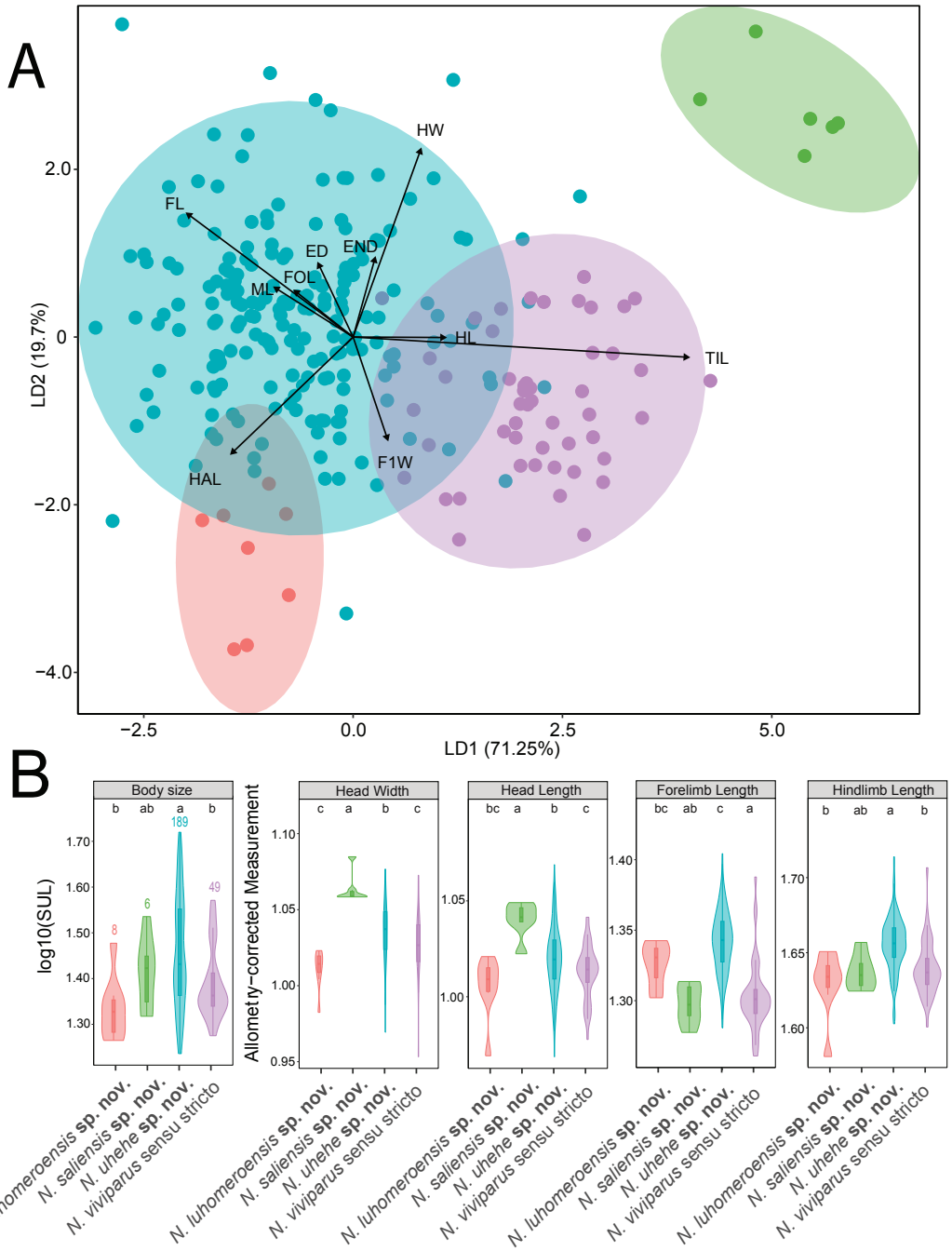

Figure 3. Morphometric differences within the *N. viviparus* complex. **A** Ordination biplot of Linear Discriminant Analysis. Arrows show the top ten loadings of the LDA, colours refer to the different species and ellipses show 95% confidence intervals of centroids. Loadings abbreviations refer to morphological measurements. **B** Box plots showing body size (snout-urostyle length) and allometry-corrected differences in head width and length and fore and hind-limb lengths. Sample sizes per species are indicated above boxplots for body size (same for all measurements) and letters show statistical groupings of post-hoc Tukey tests ($p<0.05$).

Taxonomic Treatments

Nectophrynoides viviparus (Tornier, 1905)

Suggested English common name. Southern Highlands glandular tree toad.

Suggested Kiswahili common name. Chura manundu wa nyanda za juu kusini.

Taxonomic remarks. In the following, we provide a re-description of *Nectophrynoides viviparus* sensu stricto as revealed by our museomics analysis to comprise the clade from the Southern Highlands; it supersedes previous redescriptions by e.g., Loader et al. (2009), which included non-conspecific members of the complex and cannot therefore be used to distinguish among members of that clade. However, we do not provide a redescription of the lectotype, which was adequately described by Loader et al. (2009).

Lectotype. An adult female specimen in the Museum für Naturkunde, Berlin, Germany, ZMB 21775 collected 2nd of June 1900 in Ngosi (Ngozi) Crater, Poroto Mountains, Mbeya Region, Tanzania (approximate coordinates: –9.00, 33.56), also known as "Kratersee des Nyisilvulkans" on the original label, by Friedrich Fülleborn (Fig. 7A).

Paralectotypes. Large series of subadult and adult specimens in the Museum für Naturkunde, Berlin, Germany, ZMB 71524 and 71525 with the same collection data as the lectotype. ZMB 21784, 21788, 25296, 71527 and 71528 collected in "Daressalaam" by Dr. Emil Werth. ZMB 25297 collected in "Amani" by Prof. Dr. Julius Vosseler. ZMB 25261, 25268, 25312, 71529, 71530, 71535 and 71536 collected in "Südliches Deutsch-Ostafrika" (southern Tanzania), ZMB 71187–95, 78704–9 and 78798–803 collected between 26th and 27th of October 1899 in "Rugwe (D.O.A.)" (Rungwe, Deutsch-Ostafrika), and ZMB 84908, 84909 and 84910 collected between 26th and 27th of October 1899 in "Rugwe-Gebirge", Tanzania by Friedrich Fülleborn.

Type specimen remarks. One specimen in the Natural History Museum, London, UK, BMNH 1947.2.1945 collected by Friedrich Fülleborn without collection data, is part of the original type series. One specimen in the American Museum of Natural History (AMNH), New York City, New York, USA, AMNH A23562 collected in "Daressalaam", Tanzania by Dr. Emil Werth, is part of the original type series of *Nectophryne werthi* (Nieden 1911; Loader et al. 2009).

Nota bene. The paralectotypes ZMB 21784, 21788, 25296, 25297, 71527 and 71528 are also co-types of *Nectophryne werthi* (Nieden 1911; Loader et al. 2009) and are not conspecific with the rest of the type series of *N. viviparus* sensu stricto (Fig. 1).

Revised definition. A member of the *Nectophrynoides viviparus* species complex based on overall body proportions, glandular limbs and large parotoid glands (Fig. 4), as well as genetic affinities based on mitochondrial markers (Fig. 1). This species is characterised by the unique combination of the following set of characters: (1) distinct glandular masses on limbs; (2) medium body size (adult SUL 18.8–37.2 mm, mean 24.76 ± 4.75 mm); (3) fingers slender with rounded discs; and (4) parotoid gland fusiform and widest above arm insertion.

Revised diagnosis. *Nectophrynoides viviparus* sensu stricto is easily distinguished from *N. asperginis*, *N. cryptus*, *N. frontierei*, *N. laevis*, *N. laticeps*, *N. minutus*, *N. paulae*, *N. poyntoni*, *N. pseudotornieri*, *N. tornieri*, *N. vestergaardi* and *N. wendyae* by having distinct glandular masses on limbs (versus indistinct or absent).

Preservation status. The lectotype is in fair condition, although showing signs of discolouration, softness and other 'old' specimen attributes. The paralectotypes range

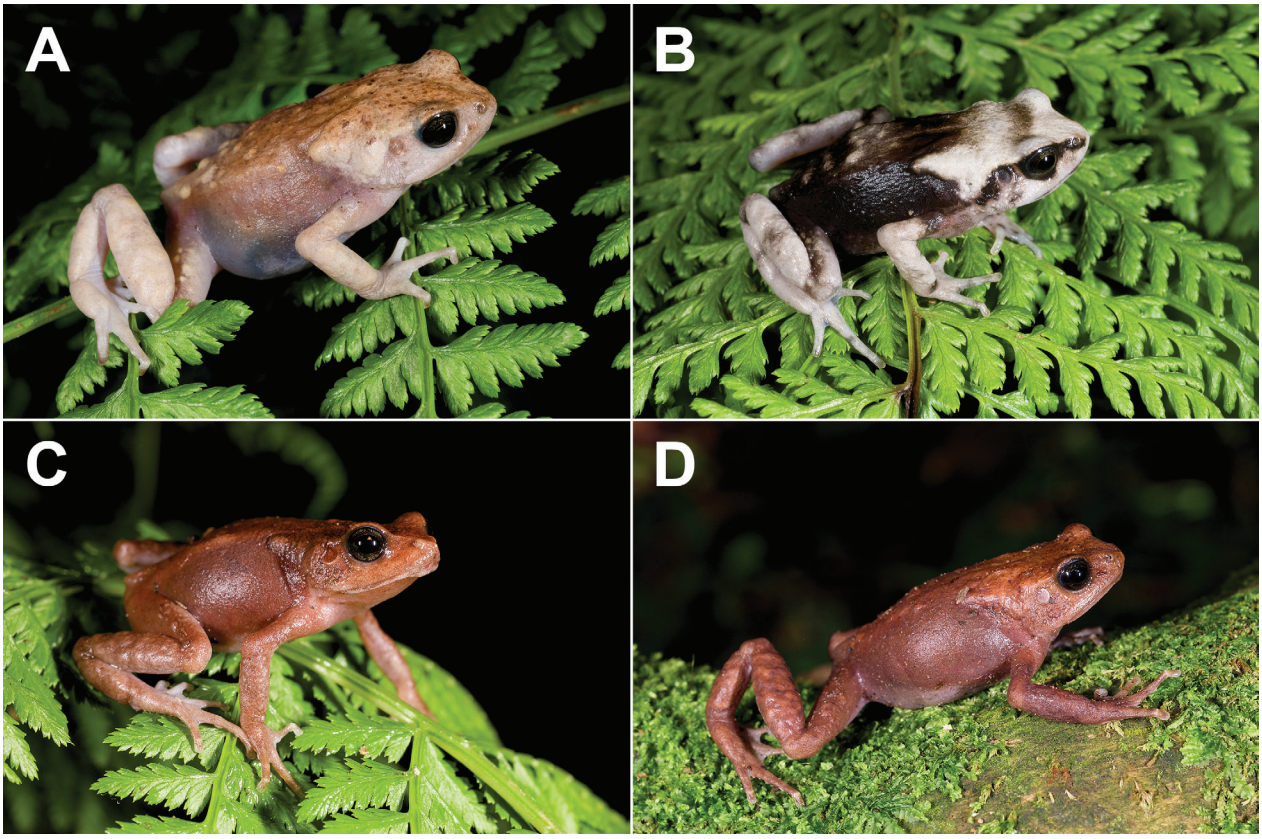

Figure 4. Intraspecific variation within *Nectophrynoides viviparus* sensu stricto in life. **A** Adult female MTSN 9407; **B** adult MTSN 9406; **C**, **D** adult MTSN 9383. Photographs from Mdandu, Livingstone Mountains by Michele Menegon.

Table 3. Uncorrected pairwise distances in the 16S rRNA barcode region. Values below the diagonal are ranges. Values on the diagonal are intraspecific ranges. Values above the diagonal are mean ± standard deviation.

	***N. viviparus* sensu stricto (n = 3)**	***N. luhomeroensis* sp. nov. (n = 2)**	***N. uhehe* sp. nov. (n = 9)**	***N. saliensis* sp. nov. (n = 5)**
N. viviparus sensu stricto	0–0.561	3.828 ± 0.276	0.374 ± 0.166	3.190 ± 0.118
N. luhomeroensis **sp. nov.**	3.589–4.067	0–0.478	3.511 ± 0.300	4.616 ± 0.279
N. uhehe **sp. nov.**	3.846–4.317	3.125–4.077	0–0.481	4.814 ± 0.211
N. saliensis **sp. nov.**	3.110–3.349	4.317–5.036	4.567–5.288	0–0.718

from bad to good condition, the specimens in bad condition are dehydrated, and some have had incisions made on the thigh or inguinal region.

Genetics. MTSN 9365 and 9383 have been successfully sampled and sequenced (Liedtke et al. 2016). Museomics were done on the following name-bearing types ZMB 21775, 21784, 21788, 25261, 25296, 25297, 25312, 71187 and 71193. *Nectophrynoides viviparus* sensu stricto is genetically distinct according to Liedtke et al. (2016), who used species delimitation approaches (specifically bGYMC) to examine current bufonid diversity against undescribed diversity. In their analysis, *N. viviparus* sensu stricto was genetically distinct and identified as "*Nectophrynoides viviparus*". MTSN 9365 and 9383 are at least 3.11% genetically different in partial (ca. 562 bp) 16S rRNA from all other *Nectophrynoides*, with the closest relative being *N. saliensis* **sp. nov.** (see Table 3). This is rather at the inter-specific level than the infra-specific (population) level; the intra-specific distance between sequenced specimens is 0–0.561%.

Bioacoustics. The call analysis was carried out on a single audio file consisting of 7 calls with a mean of 13.43 pulses per call in each audio file. The call was recorded 26th of January 2011 in Mdandu, Livingstone Mountains, the Southern Highlands, Tanzania (–9.7719, 34.7867) at around 2100 m above sea level (a.s.l.) by Michele Menegon near a stream in a closed canopy montane rainforest (Fig. 5). The calls are not associated with any known specimen. However, to our knowledge, this is the only audio file from the Southern Highlands populations, and we therefore cautiously assume that this audio file is a suitable representative of *Nectophrynoides viviparus* sensu stricto. Audio file containing calls of this species deposited online (https://doi.org/10.5281/zenodo.17277236).

The calls consist of a sequence of 12–15 pulses per call (Fig. 6A). The mean call duration is 0.23 ± 0.03 s (range: 0.20–0.27 s), with a mean call interval of 16.00 ± 5.29 s (7.36–22.27 s). Each call contains a mean of 13.43 ± 1.27 pulses (12–15), with a mean pulse duration of 0.017 ± 0.001 s (0.005–0.019 s). The mean dominant

Figure 5. Habitat of *Nectophrynoides viviparus* sensu stricto in Mdandu, Livingstone Mountains F.R. Photograph by Michele Menegon.

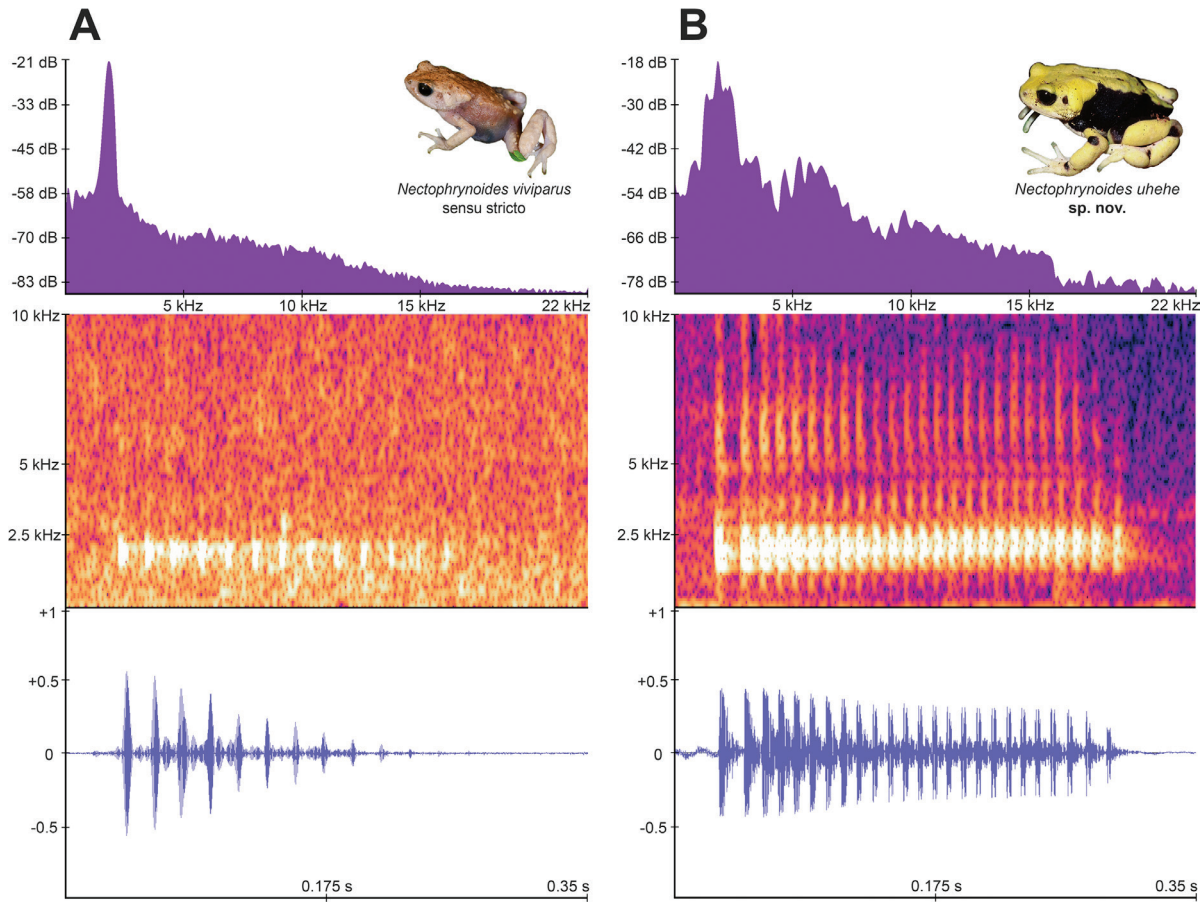

Figure 6. Pulse train arrangement and frequency landscape of **A** *Nectophrynoides viviparus* sensu stricto from Mdandu, Livingstone Mountains; **B** *N. uhehe* **sp. nov.** from Uzungwa Scarp N.F.R., Udzungwa Mountains. Plots from top to bottom show: frequency spectrum (FFT function width = 128, linear scale, Hann Window); spectrogram; and waveform (pulse train). Spectrogram and waveform are shown over the duration of a call (0–0.35 s).

frequency is 1877.71 ± 54.62 Hz (1809–1979 Hz). The call structure is illustrated in a spectrogram and waveform in Fig. 6A. The male advertisement call is monophasic consisting of pulse trains of similar proportions. The first pulse has the highest intensity followed by a series of pulses that slowly decrease in intensity (Fig. 6A). The audio file used for this analysis was sound polluted by a nearby stream. For statistical comparisons between *Nectophrynoides viviparus* sensu stricto and *N. uhehe* **sp. nov.** see Table 2. For visual comparisons between *N. viviparus* sensu stricto and *N. uhehe* **sp. nov.** see Fig. 6. More behaviour studies and recordings need to be made in the field to rule out certain factors that could shape the call, such as areas of close vicinity with a high competition between males, stress calls, and simplified communicational calls.

Etymology. The Latin adjective *viviparus*, meaning 'bearing live offspring'. The suggested common name is a reference to the distribution of this species across the Southern Highlands, its glandular skin, and semi-arboreal lifestyle. One of the previous common names of this species was "Morogoro tree toad", but this is no longer a valid representation of this species since it is not considered to occur in the Morogoro Region or District of eastern Tanzania.

Habitat and life history. As mentioned in Loader et al. (2009), and with additional collection data from ZMUC specimens, collectors have found this species from approximately 1800 to 2800 m a.s.l. The species (here referring to *N. viviparus* sensu stricto) has been associated with a range of different habitats such as wet, open, closed, primary, secondary and disturbed forests, ericaceous heathland, montane grassland and bamboo forests. The original description, and observation of toadlets, suggest that this species is ovoviviparous, as in its congeners.

Nectophrynoides luhomeroensis sp. nov.

https://zoobank.org/1CA62E89-B18C-43D2-B929-93084-C41031C

Suggested English common name. Luhomero glandular tree toad.

Suggested Kiswahili common name. Chura manundu wa mlima Luhomero.

Taxonomic remarks. This species has previously been referred to as "*Nectophrynoides* sp06" by Liedtke et al. (2016).

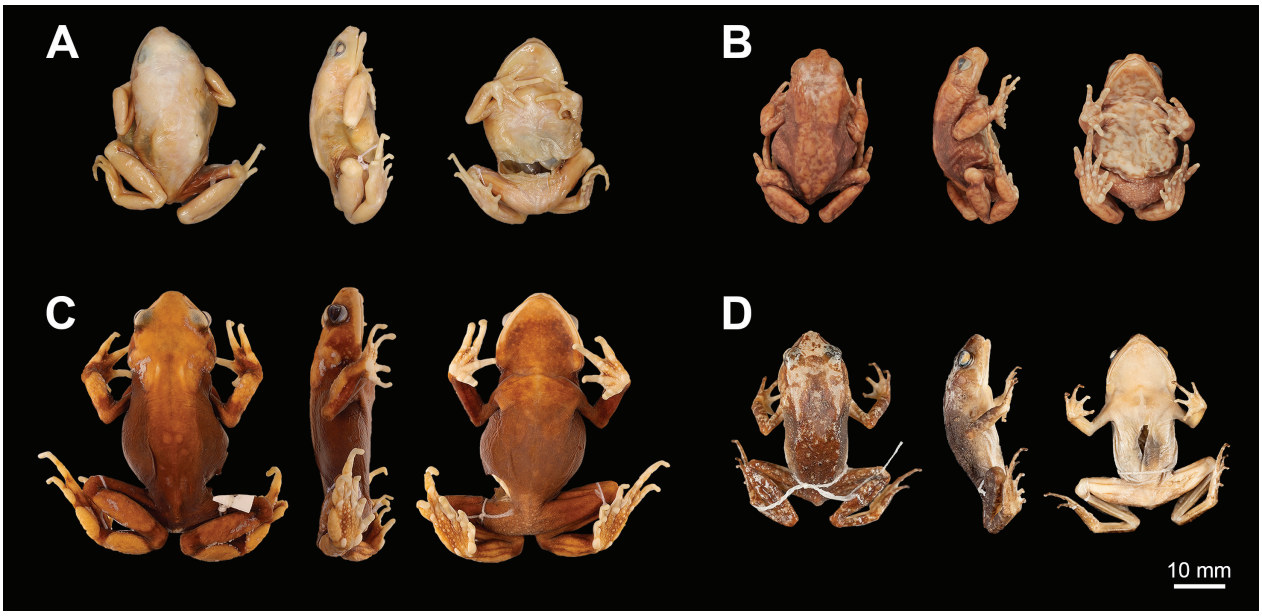

Figure 7. Comparative photographs of lectotype and holotypes. For each specimen, dorsal (left), lateral (centre), and ventral (right) views are shown. **A** Lectotype of *Nectophrynoides viviparus* sensu stricto (ZMB 21775); **B** holotype of *N. luhomeroensis* **sp. nov.** (BM 1983.6; KMH 2438); **C** holotype of *N. uhehe* **sp. nov.** (ZMUC R131391; M000044); **D** holotype of *N. saliensis* **sp. nov.** (MUSE 13758; KMH26644). Scale bar: 10 mm.

Holotype. An adult female specimen in the Natural History Museum, London, United Kingdom, BM 1983.6 (KMH 2438) collected on the 25th of October 1987 in Luhomero Mountains, Udzungwa Mountains National Park, Udzungwa Mountains, Iringa Region, Tanzania (approximate coordinates: –7.78, 36.60) at 2500 m a.s.l. by Jan Kielland (Fig. 7B).

Paratypes. Series of seven subadult and one juvenile specimens in the Museo Tridentino di Scienze Naturali, Trento, Italy, MTSN 8311, 8312, 8397, 8401, 8404, 8405, 8408 and 8409, collected on the 15th of September 2004 in Luhomero Mountains, Udzungwa Mountains National Park, Udzungwa Mountains, Iringa Region, Tanzania (–7.6965, 36.5722) at 2200 m a.s.l. by Michele Menegon.

Definition. A member of the *Nectophrynoides viviparus* species complex based on overall body proportions, glandular limbs and large parotoid glands (Fig. 8), as well as genetic affinities based on mitochondrial markers (Fig. 1). This species is characterised by the unique combination of the following set of characters: (1) distinct glandular masses on limbs; (2) medium body size (adult SUL 18.4–30.0 mm, mean 21.79 ± 3.77 mm); (3) expanded, rounded finger and toe tips with small discs; (4) parotoid gland rhomboid and slightly pointed posteriorly; (5) relative head width (HW/SUL) 0.35–0.39; and (6) relative head length (HL/SUL) 0.33–0.37.

Diagnosis. *Nectophrynoides luhomeroensis* **sp. nov.** is easily distinguished from *N. asperginis*, *N. cryptus*, *N. frontierei*, *N. laevis*, *N. laticeps*, *N. minutus*, *N. paulae*, *N. poyntoni*, *N. pseudotornieri*, *N. tornieri*, *N. vestergaardi* and *N. wendyae* by having distinct glandular masses on limbs (versus indistinct or absent).

Nectophrynoides luhomeroensis **sp. nov.** is distinguishable from *N. viviparus* sensu stricto by its slightly smaller body size (SUL 18.4–30.0 mm vs 18.8–37.2 mm), finger- and toe-tips more expanded and less slender and rounded, and parotoid glands rhomboid shaped, slightly pointed posteriorly (Fig. 9B) (vs fusiform shaped and rounded posteriorly [Fig. 9A]). For comparison to the other two new species described herein, refer to the diagnoses of the respective taxa, below.

Generalised description. A medium-sized (SUL: 30 mm) and robust *Nectophrynoides* with relatively short, muscular, and glandular limbs. The snout is triangular with a rounded tip and extends slightly beyond the upper lip. The canthus rostralis is slightly concave and flattened. The tympanum is distinct. The parotoid glands are distinct and continuous with the dorsal orbits. The parotoid glands extend from the posterior end of the eyes to above the arm insertion in the scapular region forming a rhomboid shape (Fig. 9B). The body has irregular glandular patches scattered across the dorsal and lateral surfaces. The limbs with distinct glandular masses. The length of the foot is greater than the length of the tibia. The hands and feet with rudimentary webbing. The finger and toe-tips are expanded and rounded.

In preserved and alive specimens, the colouration and patterning are highly variable (Fig. 8). Preserved specimens have a cream to dark tawny brown ground colour with darker lateral flanks. The glandular masses are lighter tawny brown with caramel brown patterning or cream brown with little to no patterning.

Description of holotype. BM 1983.6 (KMH 2438), an adult female. There are large yolky eggs visible through the abdomen. All measurements are given in mm. A me-

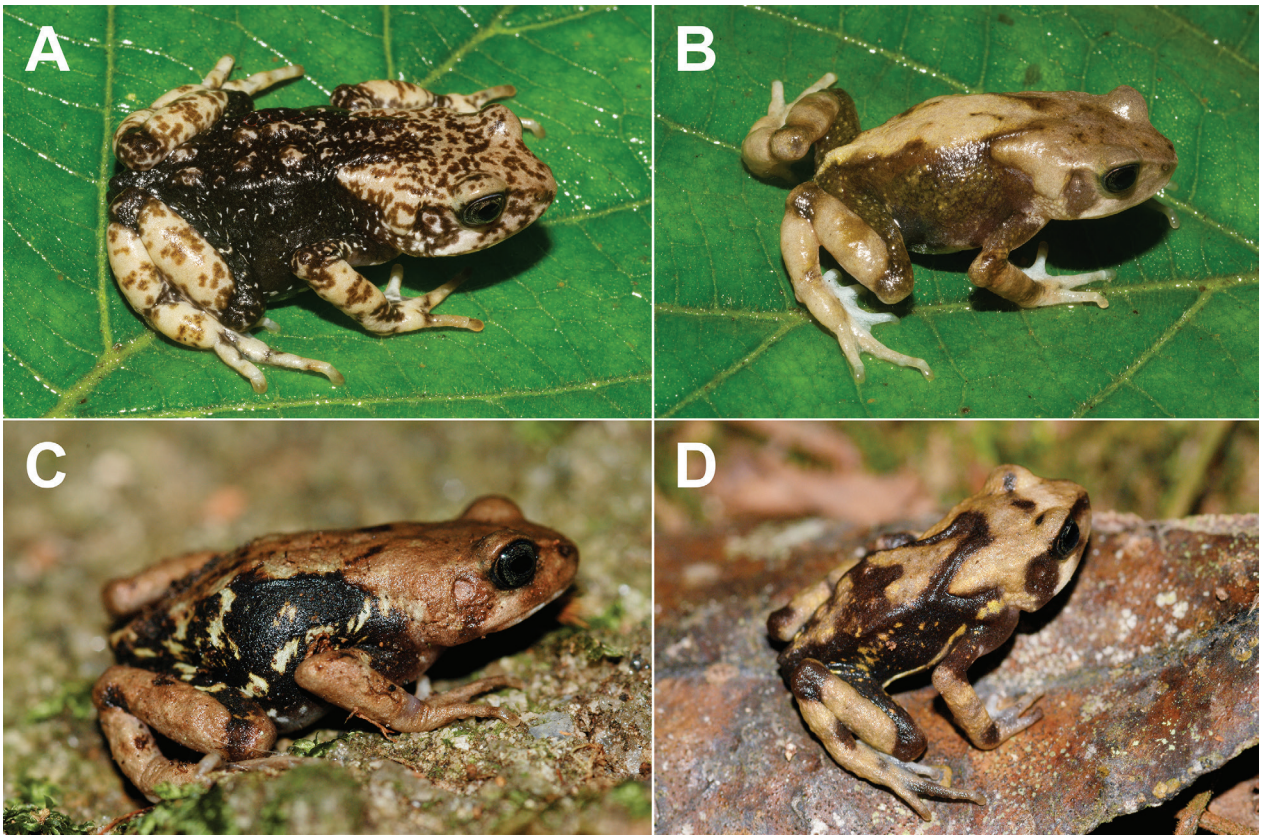

Figure 8. Intraspecific variation within *Nectophrynoides luhomeroensis* **sp. nov.** in life. **A** Adult JVL 1291; **B** adult JVL 1292; **C** subadult paratype MTSN 8312; **D** subadult paratype MTSN 8401. Photographs from Luhomero Mountains, Udzungwa Mountains; **A**, **B** by John V. Lyakurwa; **C**, **D** by Michele Menegon.

dium-sized and robust specimen (SUL: 30.0, SVL: 30.7). Width of head (HW: 10.7) almost equal to length of head (HL: 10.9). Lower jaw rounded in dorsal and ventral profile with a very slightly blunted snout. Triangular snout slightly rounded anteriorly. In lateral profile, anterior end of snout level with bottom of eye, and inclines to upper jaw. Nostrils situated on either side of snout, at level of eye centre (ND: 2.7), and clearly visible dorsally. Eyes relatively large and bulging in dorsal profile (ED: 3.4). Distance between eye and naris (END: 2.1) greater than distance between naris and tip of snout (NSD: 1.8). In lateral profile, eye and dorsal orbit are continuous with anterior end of snout to scapular region. Canthus rostralis flattened and loreal region slightly concave from top of canthus rostralis to edge of upper jaw. Canthus rostralis visible in dorsal profile. Tympanum and tympanic annulus distinct and rounded. Horizontal diameter of tympanum (TYMP: 1.1) roughly 1/3 of horizontal diameter of eye. Forelimbs muscular and relatively short. Forearm longer than humerus (FOL: 8.0, HUL: 5.9), hand longest (HAL: 9.4). Outer metacarpal tubercle length greater than width (OMCL: 1.9, OMCW: 1.5), inner metacarpal tubercle shortest (IMCL: 1.0). First and third fingertip almost equally expanded (F1W: 0.9, F3W: 0.9). Hindlimbs muscular and relatively long. Tibia and thigh almost equal in length (TIL: 12.3, THL: 12.7), roughly 1/3 longer than metatarsus (ML: 8.0), foot longest (FL: 14.0). Outer metatarsal tubercle length (OMTL: 1.4) shorter than inner metatarsal tubercle (IMTL: 2.1). First toe tip less expanded (T1W: 0.8) than fourth toe tip (T4W: 0.9). Hindlimbs more than twice as long as forelimbs (HIL: 47.0, FORL: 23.3).

Skin texture smooth on glandular and non-glandular surfaces. Dorsal head and dorsum to cloacal region glandular with small pores. Dorsal orbit glandular with medium pores. Dorsum with irregular, large circular glandular masses. Dorsal surface of limbs with glandular masses. Humerus and femur with irregular glandular masses. Forearm, hands, tibia, metatarsus and feet have slightly swollen glandular masses with large pores. Parotoid glands paired and continuous with dorsal orbit. Parotoid glands with large pores and spongy texture. Parotoid glands situated from posterior to eye to scapular region above arm insertion. Parotoid glands rhomboid shaped, widest posterior to eye above angle of jaw and narrows to a slightly pointed shape in scapular region above arm insertion. Parotoid glands extend to lateral surface of tympanic region posterior to tympanum and narrows before arm insertion. Lateral surface of head consists of irregular patches of glandular and non-glandular skin. Posterior and inferior surface of tympanum to posterior end of eye with 10–15 small to medium glandular masses each with a small translucent spine. Flank with glandular patches. Ventral surfaces non-glandular except for femoral area with small, raised bumps. Fingers and toes slender with slightly expanded and rounded digit tips. Hands and feet with distinct tubercles that are raised from the skin. Hands and feet with rudimentary webbing. Feet slightly

more webbed extending slightly beyond the first subarticular tubercles.

Dorsal ground colour tawny brown. Head and dorsum with caramel brown patches and spots. Dorsum and femur with tawny brown circular raised glandular bumps without patterning. Parotoid glands, limbs, glandular masses on limbs, hands, and feet tawny brown with caramel brown patches and spots. Femur dark tawny brown close to body and caramel towards knee. Flank ground colour caramel brown with few tawny brown spots toward dorsal margin and cream brown patterning toward ventral margin. Lateral head tawny brown with cream and caramel brown patches. Nostrils caramel brown. Abdomen, pectoral region and chin cream with tawny brown patches and spots. Ventral surface of hands and feet tawny brown with cream tubercles, fingers and toes. Ventral surface of limbs dark tawny brown. Femoral area caramel brown with tawny brown bumps.

No photographs or field notes describing colouration of holotype in life are currently known.

Variation in the species. Paratypes are smaller in body size and currently considered subadults. MTSN 8311 with dark tawny brown ground colour covering dorsal and lateral surfaces with tawny brown glands. MTSN 8405 and 8409 with cream ground colour and less distinct tympana. MTSN 8312 and 8405 with several white glandular patches and spots on lateral and dorsal surfaces. Sexual dimorphism was not observed in preserved material; females are expected to be larger than males as seen in congeners, but adult male specimens are needed to confirm this. Photographs and field notes of paratypes, and other individuals, highlight a strong variation in colouration and patterning (Fig. 8).

Preservation status. The holotype is in good condition. The paratypes are generally in fair condition but are soft-fixed, making them fragile and difficult to work with. The condition of paratype MTSN 8312 has deteriorated since measurements were taken; it was then in fair condition but is now poorer.

Genetics. Paratypes MTSN 8404 and 8405 have been successfully sampled and sequenced (Liedtke et al. 2016). *Nectophrynoides luhomeroensis* **sp. nov.** is genetically distinct according to Liedtke et al. (2016), who used species delimiting approaches (specifically bGYMC) to examine current bufonid diversity against undescribed

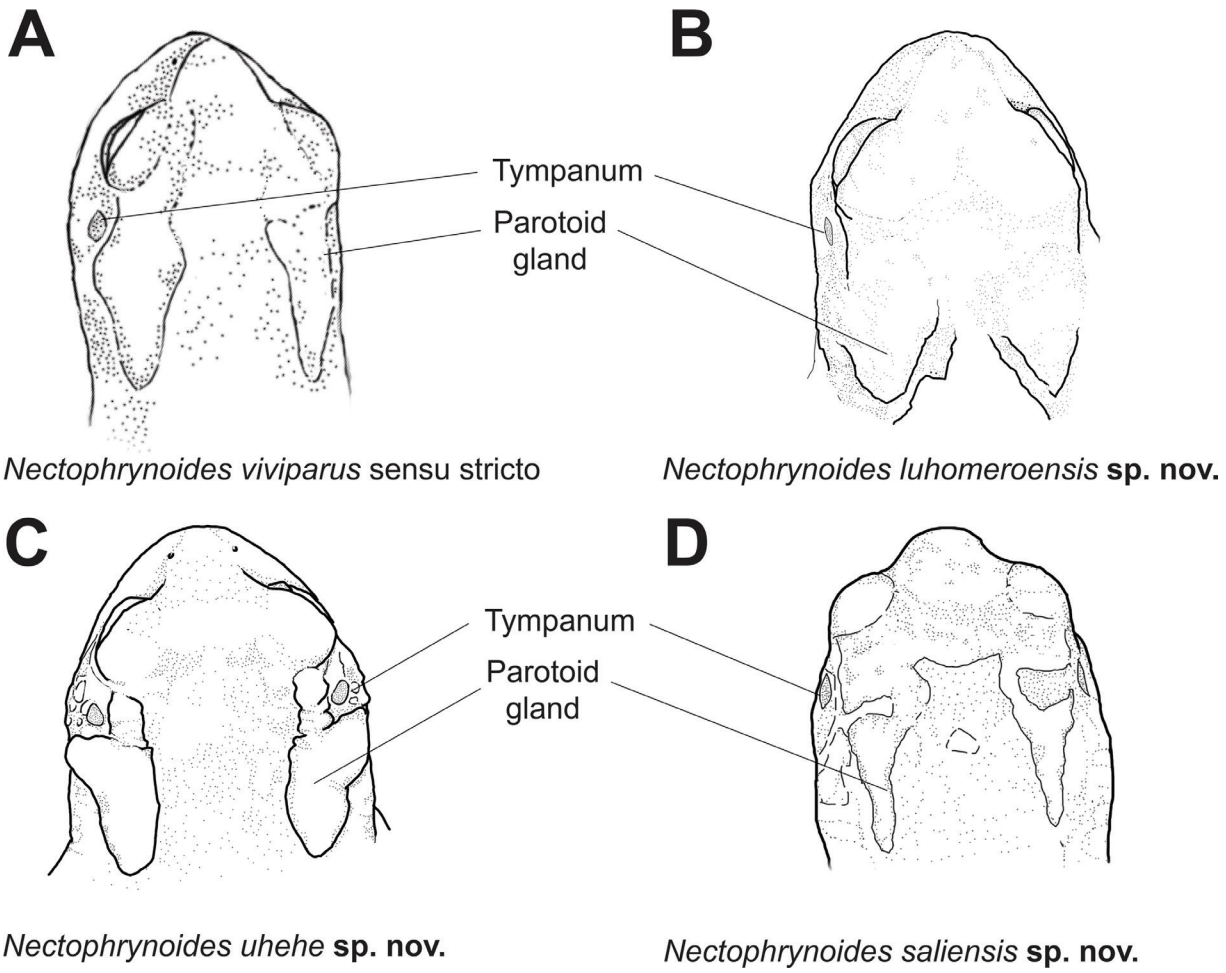

Figure 9. Comparative illustration of parotoid glands of *Nectophrynoides viviparus* sensu stricto and the new species described herein, shown in dorsal view. **A** Paralectotype of *Nectophrynoides viviparus* sensu stricto (ZMB 84909); **B** paratype of *N. luhomeroensis* **sp. nov.** (MTSN 8312); **C** paratype of *N. uhehe* **sp. nov.** (ZMUC H01955; R970529); **D** holotype of *N. saliensis* **sp. nov.** (MUSE 13758; KMH 26644). Illustrations by Simon P. Loader.

Figure 10. Habitat of *Nectophrynoides luhomeroensis* **sp. nov.** in Luhomero Mountains, Udzungwa Mountains National Park, Udzungwa Mountains. Photograph by Michele Menegon.

diversity. In their analysis, *N. luhomeroensis* **sp. nov.** was genetically distinct and identified as "*Nectophrynoides* sp06". MTSN 8405 is at least 3.13% genetically different in partial (ca. 550 bp) 16S rRNA from all other *Nectophrynoides*, with the closest relative being *N. uhehe* **sp. nov.** (see Table 3). This is rather at the inter-specific level than the infra-specific (population) level; the intra-specific distance between sequenced specimens is 0–0.478%.

Advertisement call. Not recorded.

Etymology. The species *Nectophrynoides luhomeroensis* **sp. nov.** is named after the type locality, which is Luhomero Mountains, within the Udzungwa Mountains National Park, Udzungwa Mountains, Iringa Region, Tanzania. The suggested common name is a reference to the distribution of this species across Luhomero Mountains in Udzungwa Mountains National Park, its glandular skin, and semi-arboreal lifestyle.

Habitat and life history. The specimens were hand-caught at an elevation of 2200–2500 m a.s.l., and all specimens were found in a forest and grassland mosaic (Fig. 10). Paratypes were found on the moist montane forest floor along the forest edge. The presence of large yolky eggs in the holotype, BM 1983.6, suggest that this species is ovoviviparous, as in its congeners. Two more individuals (JVL 1291–1292) were collected recently by John Lyakurwa, Glory Summay, Christina Kibwe, Anifa John and Pius Mollel in November 2023 (Fig. 8A, B). The two individuals were caught on the ground during the day in a mosaic of shrubs and grasses at 1857 m a.s.l. on the western side of the Luhomero Mountains ~2 km from the Ruipa River (–7.7563, 36.5855). The two individuals show strong resemblance both morphologically and genetically to the type specimens of *Nectophrynoides luhomeroensis* **sp. nov.** described above.

Nectophrynoides uhehe sp. nov.

https://zoobank.org/B9F04D6A-1E2D-4476-A787-DBF44C-17CDE6

Suggested English common name. Udzungwa glandular tree toad.

Suggested Kiswahili common name. Chura manundu wa milima ya Udzungwa.

Taxonomic remarks. This species has previously been referred to as "*Nectophrynoides* sp04" by Liedtke et al. (2016).

Holotype. An adult female specimen in the Natural History Museum of Denmark, Copenhagen, Denmark, ZMUC R131391 (M000044), collected 16th of December 1997 at Kihanga Stream, Uzungwa Scarp N.F.R., Udzungwa Mountains, Iringa Region, Tanzania (approximate coordinates: –8.37, 35.98) by Mette M. Westergaard (Fig. 7C).

Paratypes. Series of adults and subadults in the Natural History Museum of Denmark, Copenhagen, Denmark: Adult gravid female ZMUC R131389 (M000031),

and adults R131390 (M000036) and R131392, all with the same collection data as the holotype. Adult ZMUC H001955 (R970529), collected 26th of May 1997 in Kihanga Stream, Uzungwa Scarp N.F.R., Udzungwa Mountains, Iringa Region, Tanzania (–8.3683, 35.9783) at 1750 m a.s.l. by David C. Moyer in primary montane forest. Series of adults in the Museo Tridentino di Scienze Naturali, Trento, Italy: MUSE 5247 (MTSN 5247), 5248 (MTSN 5248) and 5249 (MTSN 5249) collected between 4th and 19th of January 1999 in Kihanga, Uzungwa Scarp N.F.R., Udzungwa Mountains, Iringa Region, Tanzania (–8.3733, 35.9786) at 1800 m a.s.l. by Michele Menegon in a closed canopy montane rain forest.

Definition. A member of the *Nectophrynoides viviparus* species complex based on overall body proportions, glandular limbs and large parotoid glands (Fig. 11), as well as genetic affinities based on mitochondrial markers (Fig. 1). This species is characterised by the unique combination of the following set of characters: (1) distinct glandular masses on limbs; (2) large body size (adult SUL 17.2–52.5 mm, mean 29.83 ± 8.61 mm); (3) expanded, rounded finger and toe tips with small discs; (4) parotoid gland not continuous with dorsal orbit, kidney shaped; (5) relative head width (HW/SUL) 0.33–0.42; and (6) relative head length (HL/SUL) 0.32–0.41.

Diagnosis. *Nectophrynoides uhehe* **sp. nov.** can be distinguished from *N. asperginis*, *N. cryptus*, *N. frontierei*, *N. laevis*, *N. laticeps*, *N. minutus*, *N. paulae*, *N. poyntoni*, *N. pseudotornieri*, *N. tornieri*, *N. vestergaardi* and *N. wendyae* by its very large body size and having large, distinct glandular masses on limbs (versus indistinct or absent).

Nectophrynoides uhehe **sp. nov.** is distinguishable from *N. viviparus* sensu stricto by its larger body size (SUL 17.2–52.5 mm vs 18.8–37.2 mm) and having more expanded, distinct limb and parotoid glands. The shape of finger- and toe tips of *N. uhehe* **sp. nov.** are more expanded than *N. viviparus* sensu stricto, which has more slender and rounded fingers. The parotoid glands are not continuous with the dorsal orbits, they are large and protruding, forming a rough kidney shape (Fig. 9C), whereas the parotoid glands of *N. viviparus* sensu stricto are continuous with the dorsal orbits, they are smaller and less protruding, forming a rough fusiform shape (Fig. 9A).

Nectophrynoides uhehe **sp. nov.** is distinguishable from *N. luhomeroensis* by a larger maximum body size (SUL 17.2–52.5 mm vs 18.4–30.0 mm) and less distinct glandular limbs, and kidney-shaped and more pronounced parotoid glands (Fig. 9C) (vs rhomboid and less pronounced; Fig. 9B).

For distinction from *N. saliensis* **sp. nov.**, refer to the diagnosis of that species, below.

Generalised description. A large and robust *Nectophrynoides* with relatively short, muscular and very glandular limbs. The snout shape is triangular with a rounded tip, extending slightly beyond the upper lip. The canthus rostralis is slightly concave and flattened. The tympanum

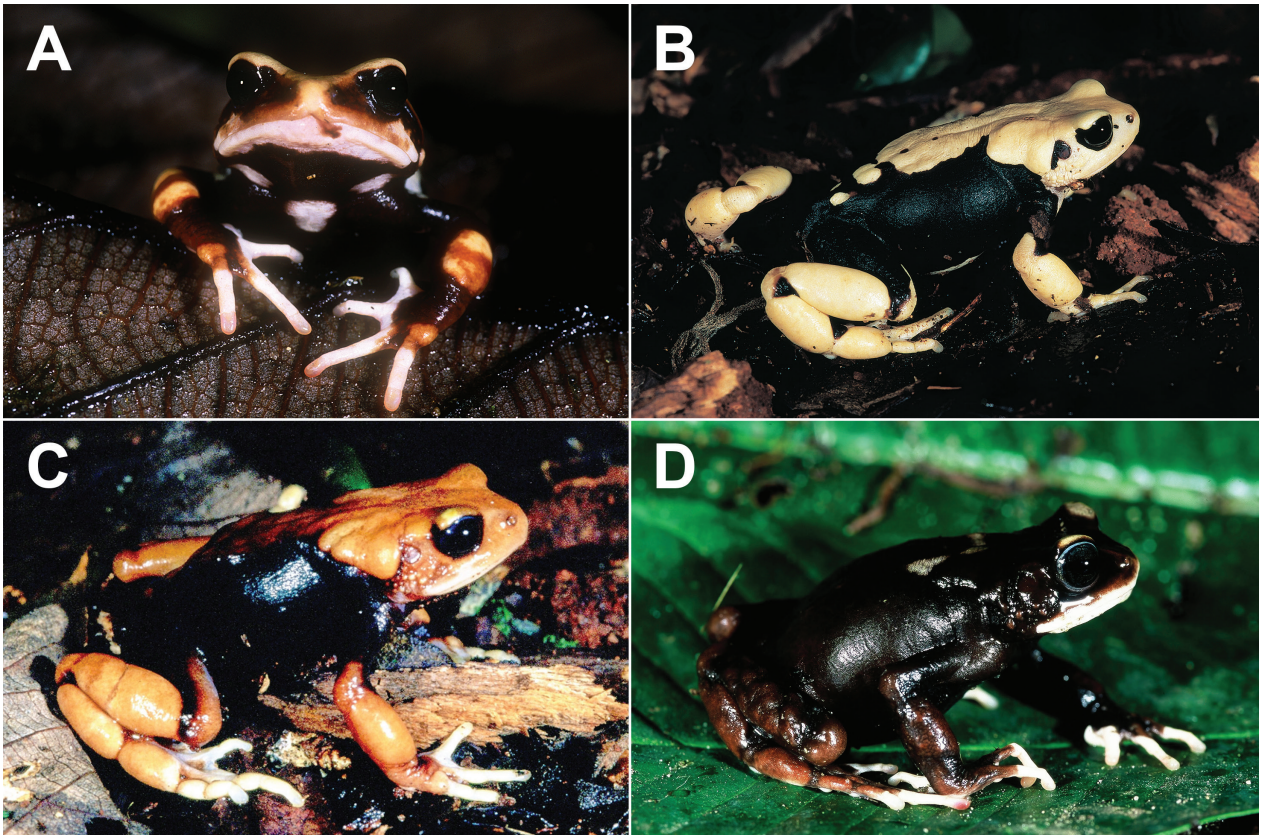

Figure 11. Intraspecific variation within *Nectophrynoides uhehe* **sp. nov.** in life. **A** Adult paratype MTSN 5247; **B**–**D** unidentified adult specimens. Photographs from Kihanga (A, B, C) and Kiolela (D) in Uzungwa Scarp N.F.R., Udzungwa Mountains by Michele Menegon.

is distinct. The parotoid glands are distinct and continuous with the dorsal orbits. The parotoid glands extend from the posterior end of the eyes to above the arm insertion in the scapular region forming a rough kidney shape (Fig. 9C). The body has medium sized irregular glandular bumps and patches scattered across the dorsal and lateral surfaces. The limbs with distinct and expanded glandular masses. The hindlimb is more than twice as long as the forelimb. The length of the foot is greater than the length of the tibia. The hands and feet with rudimentary webbing. The finger and toe-tips are expanded and rounded.

In preserved specimens, the colouration and patterning are highly variable. The ground colour is caramel to very dark tawny brown with cream to tawny brown glandular bumps and patterning. Several specimens with large amounts of patterning in different shapes and sizes of variable colours from white to dark tawny brown. The glandular masses on limbs and the parotoid glands are cream to tawny brown with no patterning, or with caramel to dark tawny brown patterning.

Description of holotype. ZMUC R131391 (M000044), an adult female. All measurements are given in mm. Large and robust specimen (SUL: 45.2, SVL: 47.7). Width of head (HW: 17.6) greater than length of head (HL: 16.7). Lower jaw rounded in dorsal and ventral profile with flattened and blunted snout. Very wide triangular snout and very slightly rounded anteriorly. In lateral profile, anterior end of snout is level with bottom of eye. Nostrils situated on either side of snout, at level of eye centre (ND: 4.3), and clearly visible dorsally. Eyes relatively large and bulging in dorsal profile (ED: 5.0). Distance between eye and naris (END: 3.3) greater than distance between naris and tip of snout (NSD: 2.4). In lateral profile, eye and dorsal orbit continuous with anterior end of snout to posterior end of eye. Canthus rostralis flattened and loreal region slightly concave from top of canthus rostralis to edge of upper jaw. Canthus rostralis visible in dorsal profile. Tympanum and tympanic annulus distinct and rounded. Horizontal diameter of tympanum (TYMP: 1.7) almost 1/3 of horizontal diameter of eye. Forelimbs muscular and relatively short. Forearm longer than humerus (FOL: 11.7, HUL: 8.7), hand longest (HAL: 15.0). Outer metacarpal tubercle length almost equal to width (OMCL: 2.3, OMCW: 2.4), inner metacarpal tubercle shortest (IMCL: 1.8). First fingertip less expanded (F1W: 1.1) than third fingertip (F3W: 1.3). Hindlimbs muscular and relatively long. Tibia and thigh almost equal in length (TIL: 19.6, THL: 19.9), almost twice as long as metatarsus (ML: 11.6), foot longest (FL: 24.3). Outer metatarsal tubercle length (OMTL: 2.2) shorter than inner metatarsal tubercle (IMTL: 2.9). First and fourth toe tip equally expanded (T1W:1.2, T4W: 1.2). Hindlimbs more than twice as long as forelimbs (HIL: 75.3, FORL: 35.4).

Skin texture smooth on glandular and non-glandular surfaces. Dorsal head glandular with small pores. Dorsal orbits glandular with large pores. Dorsum with large, irregular, circular glandular bumps. Dorsal surface of limbs with distinct glandular masses. Humerus and femur with irregular glandular masses. Forearm, hands, tibia, metatarsus and feet have distinct, swollen glandular masses with large pores. Parotoid glands paired and continuous with dorsal orbits. Parotoid glands with large pores and spongy texture. Parotoid glands situated from posterior to eye to scapular region above arm insertion. Parotoid glands rough and asymmetrical kidney shape, widest posterior to tympanum above angle of jaw and narrows to a point above arm insertion. Parotoid glands extend to lateral surface of tympanic region posterior to tympanum and narrows before arm insertion. Lateral head consists of irregular patches of glandular and non-glandular skin. Canthus rostralis has glandular skin with small pores. Posterior and inferior surface of tympanum to posterior end of eye has 25 medium to large glandular masses each with a small translucent spine. Flank without glandular patches. Ventral surfaces non-glandular except femoral area with small, raised bumps. Fingers and toes stout with expanded and rounded digits. Hands and feet with distinct, raised tubercles and rudimentary webbing. Feet slightly more webbed extending slightly beyond the first subarticular tubercles.

Dorsal ground colour tawny brown. Head caramel brown. Dorsum tawny brown with large caramel brown raised circular glandular masses. A dark tawny brown indistinct and broken dorsal stripe runs from snout to cloaca. Tawny brown glandular masses on humerus and femur. Parotoid glands and glandular masses on limbs, hands and feet cream to caramel brown. Fingers and toes cream. Flank dark tawny brown. Lateral head tawny brown with cream and caramel brown patches. Nostrils caramel brown. Canthus rostralis, upper and lower lip cream. Dorsal orbits bluish ash grey. Abdomen dark tawny brown. Pectoral region and chin tawny brown with indistinct cream and caramel brown spots. Ventral surface of hands and feet tawny brown with cream tubercles, fingers and toes. Ventral surface of forelimbs dark tawny brown and hindlimbs tawny brown. Femoral area tawny brown with caramel brown bumps.

No photographs or field notes describing colouration of holotype in life are currently known.

Variation in the species. MUSE 5247, 5248 and 5249 with irregular patterns and spots covering dorsal and ventral surfaces with white blotches on head, chin, back, abdomen, flank, limbs, hands and feet. ZMUC R131389, R131390 and R131392 are smaller (SUL: 22.79–26.94 vs 45.22) with more slender fingers and toes, and less distinct glandular masses on limbs, hands and feet. There is a large variety in colouration and patterning between individuals (Fig. 11). Sexual dimorphism was not observed in preserved material, but females are expected to be larger than males, like congeners.

Preservation status. The holotype and paratypes are in good condition.

Genetics. Paratypes MUSE 5248 and 5249 have been successfully sampled and sequenced (Liedtke et al. 2016). *Nectophrynoides uhehe* **sp. nov.** is genetically distinct according to Liedtke et al. (2016), who used

species delimiting approaches (specifically bGYMC) to examine current bufonid diversity against undescribed diversity. In their analysis, *N. uhehe* **sp. nov.** was genetically distinct and identified as "*Nectophrynoides* sp04". The specimen used in the analysis done in Liedtke et al. (2016) was MHNG 2609.071 (field number TZ-088), which is effectively 100% genetically identical to paratype MUSE 5249. MUSE 5249 is at least 3.13% genetically different in partial (ca. 550 bp) 16S rRNA from all other *Nectophrynoides*, with the closest relative being *N. luhomeroensis* **sp. nov.** (see Table 3). This is rather at the inter-specific level than the infra-specific (population) level; the infra-specific distance between sequenced specimens is 0–0.481%.

Bioacoustics. The call analysis was carried out on two audio files both consisting of 11 calls with a mean of 25.27 pulses per call in each file. The two calls were recorded in January 1999 in Site 5, Mkaja, Uzungwa Scarp N.F.R., Udzungwa Mountains, Tanzania (–8.3420, 35.9671) at 1800 m a.s.l. by Michele Menegon in an open montane wetland consisting of an ecotone between bamboo forest and open wetland (Menegon and Salvidio 2005). The two calls are not associated with any known specimens. However, the two calls were recorded nearby to the type locality. Therefore, we assume that these calls are suitable representatives of *Nectophrynoides uhehe* **sp. nov.** A singular audio file containing calls of this species deposited online (https://doi.org/10.5281/zenodo.17277236).

The two calls were practically identical in all aspects of their call parameters. We cannot confirm whether these are of one or two individuals. The calls consist of a sequence of 16–30 pulses per call (Fig. 6B). The mean call duration is 0.28 ± 0.03 s (range: 0.22–0.32 s), with a mean call interval of 3.24 ± 1.27 s (2.10–5.95 s). Each call contains a mean of 25.27 ± 3.66 pulses (16–30), with a mean pulse duration of 0.011 s +/– 0.001 s (0.011–0.014 s). The mean dominant frequency is 1891.64 ± 7.17 Hz (1883–1904 Hz). The call structure is illustrated in a spectrogram and waveform in Fig. 6B.

The male advertisement call is monophasic consisting of pulse trains of similar proportions. The first pulse has the highest intensity followed by a series of pulses that slowly decreases in intensity. The two recordings show remarkable resemblance, with almost identical results. It is worth noting that both call series begin by a pulse train that has a significantly lower number of pulses. The background noises from the habitat, people talking, and other interferences indicate that these two individual call series were taken separately and illustrate a remarkable consistency within the call structure of this species.

The few recordings highlight the need for further field research and analysis of the bioacoustics in the *Nectophrynoides viviparus* species complex. We can therefore only hint at the possibility that the call of *N. uhehe* **sp. nov.** is distinctive, and easily distinguishable from other *Nectophrynoides* species in the field. The call of the new species is distinct from *N. viviparus* sensu stricto which has fewer pulses in each pulse train (mean is ~13 pulses), and a longer duration between calls (Fig. 6A). For the statistical analysis between the two species see Table 2. However, the low number of recordings result in uncertainty regarding the actual difference between calls. More behaviour studies and recordings need to be made in the

Figure 12. Habitat of *Nectophrynoides uhehe* **sp. nov.** in Kihanga, Uzungwa Scarp N.F.R., Udzungwa Mountains. Photograph by Michele Menegon.

field to rule out certain factors that could shape the calls such as close vicinity with high competition between males, stress calls, and simplified communicational calls.

Etymology. The species *Nectophrynoides uhehe* **sp. nov.** is named in honour of the indigenous Hehe tribe, who live in villages surrounding the forests where the species occurs, for their support/involvement in herpetological surveys in the area. The Swahili word "uhehe" indicates something with affinity to the Hehe tribe. The suggested common name is a reference to the distribution of this species across the Udzungwa Mountains, its glandular skin, and semi-arboreal lifestyle.

Habitat and life history. Specimens have been collected in several forest fragments across the Udzungwa Mountains, including Kigogo F.R., Kiolela F.R., Kitungulu F.R., Mufindi Scarp F.R., Uzungwa Scarp N.F.R., and Kilombero N.F.R. The type specimens were collected in closed canopy montane rainforest between 1700 and 1800 m a.s.l. near Kihanga stream inside Uzungwa Scarp N.F.R. (Fig. 12). The presence of developed toadlets inside several adult female specimens suggests that this species is ovoviviparous, as in its congeners. More recent observations are known by John Lyakurwa, Michele Menegon and Elena Tonelli in 2014–2016 at multiple locations within the Uzungwa Scarp N.F.R. from 1600 m a.s.l. Individuals were observed on the ground, around small wetlands both inside and bordering the forest, and some specimens were observed up to 2 m above the ground on understory vegetation in the moist forest inside closed canopy montane rainforest.

Nectophrynoides saliensis sp. nov.

https://zoobank.org/0E9E8F7C-8CDE-4F9F-890A-3928-26FBA403

Suggested English common name. Mahenge glandular tree toad.

Suggested Kiswahili common name. Chura manundu wa Mahenge.

Taxonomic remarks. This species has previously been referred to as "*Nectophrynoides* sp13" by Liedtke et al. (2016).

Holotype. An adult presumably female specimen in the Museo Tridentino di Scienze Naturali, Trento, Italy, MUSE 13758 (KMH 26644) collected December 2005 in Sali F.R., Mahenge Mountains, Morogoro Region, Tanzania (approximate coordinates: –8.93, 36.65) at 1050–1500 m a.s.l. by Frontier-Tanzania (Menegon et al. 2011a) (Fig. 7D).

Paratypes. Series of adults and subadults: MUSE 13754 (KMH 26637), 13755 (KMH 26638), 13756 (KMH 26639), 13757 (KMH 26641) and 13759 (KMH 26998), all with the same collection data as the holotype.

Definition. A member of the *Nectophrynoides viviparus* species complex based on overall body proportions, glandular limbs and large parotoid glands (Fig. 7D), as well as genetic affinities based on mitochondrial markers (Fig. 1). This species is characterised by the unique combination of the following set of characters: (1) indistinct glandular masses on limbs; (2) medium body size (adult SUL 20.8–34.3 mm, mean 26.30 ± 5.02 mm); (3) weakly expanded rounded finger and toe tips with minute discs; (4) parotoid gland spearhead shaped; (5) relative head width (HW/SUL) 0.40–0.43; and (6) relative head length (HL/SUL) 0.37–0.40.

Diagnosis. *Nectophrynoides saliensis* **sp. nov.** is easily distinguished from *N. asperginis*, *N. cryptus*, *N. frontierei*, *N. laevis*, *N. pseudotornieri*, *N. wendyae* by having a distinct tympanum.

The smooth dorsal surface of *Nectophrynoides saliensis* **sp. nov.** is covered with glandular patches and warts, indistinct glandular masses on limbs, and the parotoid glands form a large distinct spearhead shape, which distinguishes it from *N. laticeps*, *N. minutus*, *N. tornieri*, *N. paulae*, *N. poyntoni*, *N. vestergaardi.*

Nectophrynoides saliensis **sp. nov.** is distinguishable from *N. viviparus* sensu stricto by having indistinct limb glands. The finger- and toe-tip expansion of *N. saliensis* **sp. nov.** are also more expanded than *N. viviparus* sensu stricto, which has more slender and rounded fingers. The parotoid glands are elongated, and spearhead shaped, narrowing to a thin acuminate shape posteriorly (Fig. 9D), whereas the parotoid of *N. viviparus* sensu stricto are fusiform shaped and rounded posteriorly (Fig. 9A).

Nectophrynoides saliensis **sp. nov.** is distinguished from *Nectophrynoides luhomeroensis* by lacking distinct limb glands (vs distinct), differently shaped parotoid glands (spearhead vs rhomboid [Fig. 9D and Fig. 9B, respectively]), larger relative head size (HL/SUL 0.37–0.40 vs 0.33–0.37, and HW/SUL 0.40–0.43 vs 0.35–0.39), and from *N. uhehe* by a much smaller maximum body size (SUL 34.3 mm vs 52.5 mm), by lacking distinct limb glands (vs distinct), and differently shaped parotoid glands (spearhead vs kidney [Fig. 9D and Fig. 9C, respectively]).

Generalised description. A medium-sized (SUL: 34.3 mm) and robust *Nectophrynoides* with relatively short, muscular and slightly glandular limbs. The snout shape is triangular with a rounded tip and extending slightly beyond the upper lip. The canthus rostralis is angular. The tympanum is distinct. The parotoid glands are distinct and continuous with the dorsal orbits. The parotoid glands extend from the posterior end of the eyes to above the arm insertion in the scapular region forming a rough spearhead shape (Fig. 9D). The body has small irregular glandular bumps and patches scattered across the dorsal and lateral surfaces. The limbs with indistinct glandular masses. The length of the foot is greater than the length of the tibia.

The hands and feet with rudimentary webbing. The finger and toe-tips are expanded and rounded.

In preserved specimens, the colouration and patterning are variable. The ground colour is very dark tawny brown with ash grey glandular bumps. The glandular masses on limbs and the parotoid glands are ash grey with dark tawny brown patterning.

Description of holotype. MUSE 13758 (KMH 26644), presumably female adult. The specimen has a mid-ventral incision along the body. All measurements are given in mm. A medium-sized and robust specimen (SUL: 34.3, SVL: 35.0). Width of head (HW: 13.6) almost equal to length of head (HL: 13.1). Lower jaw rounded in dorsal and ventral profile with slightly flattened and blunted snout. Wide triangular snout slightly rounded anteriorly. In lateral profile, anterior end of snout is level with bottom of eye. Nostrils situated on either side of snout, at level of eye centre (ND: 2.3), and clearly visible dorsally. Eyes relatively large and bulging in dorsal profile (ED: 3.6). Distance between eye and naris (END: 3.0) greater than distance between naris and tip of snout (NSD: 1.5). In lateral profile, eye and dorsal orbit continuous with anterior end of snout to scapular region. Canthus rostralis angular and loreal region concave from top of canthus rostralis to edge of upper jaw. Canthus rostralis visible in dorsal profile. Tympanum and tympanic annulus distinct and rounded. Horizontal diameter of tympanum (TYMP: 1.4) less than half of horizontal diameter of eye. Forelimbs muscular and relatively short. Forearm longer than humerus (FOL: 9.3, HUL: 6.8), hand longest (HAL: 11.4). Outer metacarpal tubercle length equal to width (OMCL: 2.0, OMCW: 2.0), length of inner metacarpal tubercle shortest (IMCL: 1.5). First fingertip less expanded (F1W: 0.9) than third fingertip (F3W: 1.0). Hindlimbs muscular and relatively long. Tibia longer than thigh and metatarsus (TIL: 17.8, THL: 17.0, ML: 10.3), foot longest (FL: 18.9). Outer metatarsal tubercle length (OMTL: 1.7) shorter than inner metatarsal tubercle (IMTL: 2.6). First and fourth toe tip equally expanded (T1W:1.0, T4W: 1.0). Hindlimbs more than twice as long as forelimbs (HIL: 64.1, FORL: 27.5).

Skin texture smooth on glandular and non-glandular surfaces. Dorsal head glandular with small pores. Dorsal orbits glandular with medium pores. Dorsum with small, irregular, raised glandular bumps. Front and hind limbs with indistinct glandular masses. Humerus and femur have small irregular glandular bumps. Forearm, hands, tibia, metatarsus and feet have indistinct glandular masses with large pores. Parotoid glands paired and continuous with dorsal orbits. Parotoid glands with medium pores. Parotoid glands situated from posterior to eye to scapular region above arm insertion. Parotoid glands rough spearhead shaped, widest posterior to eye above angle of jaw and tympanum, narrowing to an acuminate shape in scapular region above arm insertion. Parotoid glands extend to lateral surfaces of tympanic region posterior to tympanum and become irregular patches of glandular and non-glandular skin. Lateral head consists of irregular patches of glandular and non-glandular skin. Posterior and inferior surface of tympanum to posterior end of eye has 10–15 small to medium glandular masses each with a small translucent spine. Flank with small, irregular, raised glandular bumps. Ventral surfaces non-glandular except for femoral area with small, raised bumps. Fingers and toes slender with slightly expanded and rounded digit tips. Hands and feet with distinct, raised tubercles and rudimentary webbing. Feet slightly more webbed extending slightly beyond the first subarticular tubercles.

Dorsal ground colour very dark tawny brown. Dorsum and flank with many ash grey, raised, glandular bumps. Dorsal head with a large ash grey glandular patch with very dark tawny brown spots. Limbs, hands and feet with ash grey glandular patches, spots and stripes. Tympanic region tawny brown with ash grey glandular patches. Lateral head ash grey. Canthus rostralis and nostrils tawny brown. Dorsal orbits bluish ash grey. Ventral surface of limbs, abdomen and chin cream. Pectoral regions cream with caramel brown patches. Ventral surfaces of hands and feet caramel brown with cream tubercles, fingers and toes. Femoral area caramel brown with cream bumps.

No photographs or field notes describing colouration of holotype in life are currently known.

Variation in the species. MUSE 13755 tawny brown dorsal ground colour with very dark tawny brown patterning on dorsal and ventral surfaces. MUSE 13755 has a less distinct tympanum. MUSE 13755 and 13759 have very slightly pointed snout tips. Sexual dimorphism was not observed in preserved material, but females are expected to be larger than males, like congeners.

Preservation status. The holotype and paratypes are in fair condition but show evidence of previous exposure to unsuitable preservation. The specimens are shrunken, stiff, and partially desiccated, with dried finger- and toe tips.

Genetics. Holotype MUSE 13758 and paratypes MUSE 13754, 13755, 13757 and 13759 have been successfully sampled and sequenced (Liedtke et al. 2016). *Nectophrynoides saliensis* **sp. nov.** is genetically distinct according to Liedtke et al. (2016), who used species delimiting approaches (specifically bGYMC) to examine current bufonid diversity against undescribed diversity. In their analysis, *N. saliensis* **sp. nov.** was genetically distinct and identified as "*Nectophrynoides* sp13". MUSE 13758 is 3.11% genetically different in partial (ca. 550 bp) 16S rRNA from all other *Nectophrynoides*, with the closest relative being *Nectophrynoides viviparus* sensu stricto (see Table 3). This is rather at the inter-specific level than the infra-specific (population) level; the intra-specific distance between sequenced specimens is 0–0.718%.

Advertisement call. Not recorded.

Etymology. The species *Nectophrynoides saliensis* **sp. nov.** is named after the location where the species was discovered, which is Sali F.R. in Mahenge Mountains, Tanzania. The suggested common name is a reference

Figure 13. Habitat of *Nectophrynoides saliensis* **sp. nov.** in Sali F.R., Mahenge Mountains. Photograph by Michele Menegon.

to the distribution of this species in the Mahenge Mountains, its glandular skin, and semi-arboreal lifestyle.

Habitat and life history. There is very limited knowledge of habitat, ecology, and behaviour of this species. The specimens were discovered by Frontier-Tanzania in a dense, low canopy submontane forest near open canopy wetlands in the northern inaccessible forests of Sali F.R. (Fig. 13). A paper published based on the work by Frontier-Tanzania in Mahenge Mountains describes Sali F.R. as a landscape consisting of miombo woodlands at lower elevation, submontane and montane forests, dry grassland, wetlands and large rocky outcrops (Menegon et al. 2011a). Little is known about the reproductive biology of this species but the presence of large, developed embryos visible through the abdomen of paratype MUSE 13757 suggests that the species is ovoviviparous, as in its congeners.

Key to the species of *Nectophrynoides*

Illustrations of selected morphological traits states (e.g., absent, indistinct, distinct) are provided in File S2.

1a The femoral area with a pigmented darkened patch of skin surrounding the interfemoral glands with light, often white spots on ventral surface, small sized species (SUL: 13–20 mm), dark tawny brown body with grainy skin texture, a long, pointed snout extending far beyond the upper lip, a distinct white upper lip, males with reddish pigment on ventral surface of throat, tympanum absent, parotoids reduced to warts on dorsal surface ..... ***N. wendyae***

1b No darkened patch of pigmented skin with light spots in femoral area, snout slightly pointed or rounded.....**2**

2a Large conical spines covering most dorsal surfaces on head, dorsal orbits, dorsum and hindlimbs with keratinized horned tips, a set of six larger spines situated symmetrically on head and shoulders, medium sized species (SUL: 17–25 mm), caramel to tawny brown body with a seasonal spiny skin texture, often with light caramel brown patterning and white dorsal stripe, slightly pointed snout, rounded digits, distinct tympanum, parotoids indistinct .....***N. paulae***

2b No large prominent conical spines with keratinized horned tips **3**

3a Tympanum absent **4**

3b Tympanum present (distinct/indistinct) **6**

4a Extensive webbing on hands and feet, parotoid glands are absent, small sized species (SUL: 12–20 mm), light golden-brown body with smooth skin texture, darker spots and patterning on dorsal and lateral surfaces, cream brown ventral surface, dark dorsolateral band, rounded snout, expanded and rounded digits .....***N. asperginis***

4b Lack of extensive webbing on hand and feet, and parotoid glands present **5**

5a Digits expanded and truncated, parotoid glands indistinct, medium sized species (SUL: 14–29 mm), caramel to dark tawny brown body with smooth skin texture and yellow lateral markings, slightly pointed snout, light band present under eye ..... ***N. pseudotornieri***

5b Digits slightly expanded and rounded, parotoids form a raised ridge more than twice as long as wide and longer than horizontal diameter of the eye, medium sized species (SUL: 25 mm), ash grey body with smooth skin texture, distinct black ventral patterning forming a rough cross shape with lines extending from abdomen to palms of hands and feet, rounded snout..... ***N. laevis***

6a Parotoids absent, tympanum indistinct, medium sized species (SUL: 18–25 mm), golden to caramel brown body with smooth skin, slightly pointed snout, digits rounded ..... ***N. frontierei***

6b Parotoids present..... **7**

7a Limbs with smooth glandular masses covering dorsal and lateral surfaces **8**

7b Limbs without smooth glandular masses covering dorsal and lateral surfaces **12**

8a Distinct webbing on feet, tympanum indistinct, parotoid glands distinct to indistinct and non-continuous with dorsal orbit, medium sized species (SUL: 18–33 mm), golden to tawny brown body with smooth to slightly glandular skin texture, indistinct glandular masses on limbs, rounded snout, digits expanded and rounded with black tips ... . ***N. cryptus***

8b Only rudimentary webbing on feet **9**

9a Indistinct smooth dark tawny brown glandular masses with ash grey patterning on limbs, parotoid glands form a rough spearhead shape (Fig. 9D), parotoids segmented in lateral profile and forms glandular patches posteriorly of tympanic region, medium sized species (SUL: 20–35 mm), dark tawny brown body with smooth skin texture, ash grey glandular masses covering dorsal surface of head and bumps on back, rounded snout, digits expanded and rounded..... ***N. saliensis***

9b Distinct glandular masses on limbs..... **10**

10a Glandular masses on limbs covering dorsal and lateral surfaces heavily expanded, fingers and toes, parotoid glands large and protrusive non-continuous with the dorsal orbit, parotoid glands form a rough kidney shape extending down into the posterior end of the tympanic region (Fig. 9C), large sized species (SUL: 20–53 mm), golden to dark tawny brown body with smooth skin texture, often with lighter coloured glandular masses on head, limbs and back, rounded snout, digits expanded and rounded..... ***N. uhehe***

10b Glandular masses on limbs covering dorsal and lateral surfaces not heavily expanded..... **11**

11a Glandular masses on limbs distinct and expanded, parotoid glands large and non-continuous with the dorsal orbit, parotoid glands form a fusiform shape slightly extending down into the posterior end of the tympanic region (Fig. 9A), medium/large sized species (SUL: 18–38 mm), usually golden to dark tawny brown body with smooth skin texture, often with lighter coloured glandular masses on head, limbs and back, rounded snout, digits slightly expanded and rounded ..... ***N. viviparus***

11b Glandular masses on limbs distinct and expanded, parotoids long and pointed towards the vent, parotoid glands form a rhomboid shape extending down into the posterior end of the tympanic region (Fig. 9B), small sized species (SUL: 16–30 mm), cream to dark tawny brown body with smooth skin texture, often with large cream glandular patches on dorsal and lateral surfaces, rounded snout, digits expanded and rounded. ***N. luhomeroensis***

12a Parotoid glands form a thin segmented ridge above tympanic region, small sized species (SUL: 13–21 mm), golden to caramel brown body with grainy skin, light band under eye present, slight pointed snout, digits expanded and rounded, tympanum distinct to indistinct..... ***N. minutus***

12b Parotoid glands form a thin ridge above tympanic region..... **13**

13a Digits very expanded and truncated, medium sized species (SUL: 16–31 mm), golden to dark caramel brown body with grainy skin texture, parotoid glands form a thin ridge above tympanic region, slightly pointed snout .... ***N. tornieri***

13b Digits not truncated..... **14**

14a Digits rounded and not expanded, medium sized species (SUL: 13–27 mm), golden to dark tawny brown body with grainy skin, often with a dark dorsal stripe, light band under eye present, parotoid glands continuous from tympanum to scapular region, slightly pointed snout ..... ***N. vestergaardi***

14b Digits expanded and rounded..... **15**

15a Parotoid glands form a sausage shape equal to the length of the dorsal orbit, dark stripe running from snout tip along canthus rostralis to scapular region, small medium sized species (SUL: 20–24 mm), golden brown to ash grey body with grainy skin, large variation in patterning often with light and dark patches covering dorsal and lateral surfaces, light band under eye present, slightly pointed snout..... ***N. poyntoni***

15b Parotoid glands form a thin ridge twice as long as the length of the dorsal orbit, small sized species (SUL: 20–26 mm), golden to caramel brown body with grainy skin, often with dark raised bumps on dorsal and lateral surfaces, slightly pointed snout..... ***N. laticeps***

Table 4. Gazetteer of known *Nectophrynoides* localities. Candidate species names are modified from Menegon et al. (2020).

Mountain(s)	Location	Species
East Usambara	Amani N.F.R.	*N. frontierei*
		N. tornieri
	Bamba Ridge F.R.	*N. tornieri*
	Kizerui	*N. tornieri*
	Kwamgumi F.R.	*N. tornieri*
	Magoroto F.R.	*N. tornieri*
	Mlinga F.R.	*N. tornieri*
	Mpanga Village F.R.	*N. tornieri*
	Mtai F.R.	*N. tornieri*
West Usambara	Mazumbai F.R.	*N. vestergaardi*
	Shume-Magamba F.R.	*N. vestergaardi*
Nguu	Kilindi F.R.	*N.* aff. *tornieri*
	Nguru North F.R.	*N.* sp.
		N. aff. *tornieri*
Nguru	Kanga F.R.	*N.* sp.
		N. aff. *tornieri*
	Mkingu N.F.R.	*N.* sp.
		N. aff. *tornieri*
		N. aff. *viviparus*
Ukaguru	Mamiwa Kisara North F.R.	*N. laticeps*
		N. paulae
Rubeho	Chugu F.R.	*N.* aff. *viviparus*
	Ilole F.R.	*N.* aff. *viviparus*
	Mafwomero F.R.	*N.* aff. *viviparus*
	Ukwiva F.R.	*N.* aff. *viviparus*
Malundwe	Mikumi N.P.	*N.* aff. *tornieri*
Uluguru	Kimboza N.F.R.	*N.* aff. *tornieri*
	Mkungwe F.R.	*N.* aff. *tornieri*
	Ruvu F.R.	*N.* aff. *tornieri*
	Uluguru N.F.R. (north)	*N. minutus*
		N. pseudotornieri
		N. aff. *viviparus*
	Uluguru N.F.R. (south)	*N. cryptus*
		N. laevis
		N. minutus
		N. pseudotornieri
		N. aff. *viviparus*
		N. aff. *viviparus* (“*werthi*”)
Udzungwa	Dabaga F.R.	*N. uhehe*
	Image F.R.	*N. uhehe*
	Kigogo F.R.	*N. uhehe*
	Kihansi Catchment	*N. asperginis*
		N. aff. *tornieri*
	Kilombero N.F.R.	*N. uhehe*
	Kiolela F.R.	*N. uhehe*
	Kitungulu F.R.	*N. uhehe*
	Mufindi Scarp F.R.	*N. uhehe*
	Udzungwa Mountains N.P.	*N. luhomeroensis*
		N. aff. *tornieri*
	Uzungwa Scarp N.F.R.	*N. poyntoni*
		N. sp. “red-lined”
		N. sp. “spiny”
		N. aff. *tornieri*
		N. uhehe
		N. wendyae
Mahenge	Sali F.R.	*N. saliensis*
		N. aff. *tornieri*
Livingstone	Mdandu F.R.	*N. viviparus*
Poroto	Poroto Ridge F.R.	*N. viviparus*
Rungwe	Mount Rungwe N.F.R.	*N. viviparus*

Table 5. Provisional conservation status of *Nectophrynoides viviparus* sensu stricto and the three new species described herein based on their estimated distribution and the threat level of defined locations. Area of Occupancy (**AOO**) and Extent of Occurrence (**EOO**) of each species, expressed in square kilometers (km^2). Calculations based on approaches applied in Cazalis et al. (2024).

Species	Range	Area Protection	AOO	EOO	Predicted Conservation Status
N. luhomeroensis	Luhomero, Udzungwa Mountains	National Park	152 km^2	162 km^2	Critically Endangered B1ab(iii)
N. saliensis	Sali, Mahenge Mountains	Forest Reserve	20 km^2	20 km^2	Critically Endangered B1ab(iii)
N. uhehe	Udzungwa Mountains	Forest Reserve to National Park	4340 km^2	5701 km^2	Vulnerable B1ab(iii)
N. viviparus	Southern Highlands	Forest Reserve to Nature Forest Reserve	304 km^2	1611 km^2	Endangered B1ab(iii), B2

Discussion

Taxonomy

Our molecular sampling of both contemporary populations and the historic type series has provided much needed clarification on the taxonomy of one of the most iconic groups of viviparous toads in Tanzania. We have provided conclusive evidence that *Nectophrynoides viviparus* is restricted to the Southern Highlands, with other populations representing distinct phylogenetic lineages (Fig. 1). The modern samples from Livingstone Mts, and Mt Rungwe, formed a well-supported clade with museomic samples of type series from various locations, including "Kratersee des Nyisilvulkans", "Rugwe, Tansania, Deutsch Ostafrika", "Südliches Deutsch Ostafrika" (Fig. 1). The specimens with less specific location data labelled as "Southern Tanzania" likely refer to specimens that have been collected somewhere in the Southern Highlands as well. Here, we have shown that the lineages found in the Southern Highlands are morphologically and genetically distant (>3%) from other lineages from southern blocks of the Eastern Arc Mountains, such as those found in the Udzungwa (*N. luhomeroensis* and *N. uhehe*) and Mahenge Mountains (*N. saliensis*). We can also confirm that populations attributed to *N. viviparus* further north in the Eastern Arc Mountains, with populations scattered across the Uluguru, Rubeho, and Nguru Mountains, are also genetically distinct from *N. viviparus* sensu stricto (Liedtke et al. 2016), and will require future taxonomic revision, which is beyond the geographic scope of this paper (see below).

Our molecular sampling from the ZMB co-type series of *Nectophryne werthi*, including locations "Daressalaam" and "Amani", clustered with specific populations attributed to *N. viviparus* in the Uluguru Mountains. This supports the view of Loader et al. (2009) that *N. viviparus* occurrence in the locations "Daressalaam" (linked to Dr. Emil Werth's collection) and "Amani" (linked to Prof. Dr. Julius Vosseler's collection) were likely incorrect given that both locations fall well outside of the known distribution of populations attributed to the *N. viviparus* species complex. The location "Amani" given by collector Prof. Dr. Julius Vosseler (1861–1933) might have been presumed, given that this was the site he was primarily located during his time in Tanzania (Tillack et al. 2021). However, Prof. Dr. Julius Vosseler collected specimens outside of Amani (Ahl 1931a, 1931b), including locations close to the Uluguru Mountains. Similarly, the botanist Dr. Emil Werth (1869–1958) was also known to collect outside of Dar es Salaam, including areas in and around the Uluguru Mountains (Tillack et al. 2021). Location data for specimens collected during this period were not always accurate, and likely inferred from the people who acquired them. This also highlights the common practice during this period in history, in which specimens were often obtained through intermediaries, rendering their collection history incomplete and difficult to reconstruct (Das and Lowe 2018). It was often the case that collections were not necessarily made by the named collectors registered in museums but often brought to "collectors" by other people who lived near field locations, where natural history specimens were collected and preserved (Ashby and Machin 2021). A known example of this, also in Tanzania, was that specimens were sometimes collected and delivered to Arthur Loveridge without detailed locality information (Barbour and Loveridge 1928; Loveridge 1951).

Our study has outlined the geographical origin of *Nectophryne werthi*, whose locations were previously given as "Amani" and "Daressalaam" in collector acquisition histories. However, the origin of these co-types is grouped with modern populations from the Uluguru Mountains, currently considered synonymous with *N. viviparus*, but representing a distinct species, which we will treat as *N.* cf. *viviparus* ("*werthi*"). As already stated, this taxon, along with populations in the Rubeho and Nguru Mountains requires further taxonomic research (Liedtke et al. 2016), which will be addressed in a separate study (Lyakurwa et al. in prep.).

Conservation

Several species of *Nectophrynoides* have experienced decline in their habitats, and consequently in population numbers, with one species, *N. asperginis* Poynton et al. 1999, having recently gone extinct in the wild (Weldon et al. 2020). Other *Nectophrynoides* species have had few, if any, sightings since their discovery, e.g., *N. poyntoni* Menegon et al., 2004. These examples highlight the potential vulnerability of this group. In the case of the species described in this study, "*N. viviparus*" is currently listed under the category "Least Concern" (**LC**) on the

International Union for Conservation of Nature's (**IUCN**) Red List (IUCN 2025). This was based on the estimated area of occupancy of 2399 km^2, and estimated extent of occurrence of 46,287 km^2, which placed it outside of any conservation concern. The status relied on the concept of the species being widely distributed, i.e., large distribution, and present at more than 6 localities (IUCN 2025). Our study shows that *N. viviparus* sensu stricto is restricted to the Southern Highlands in several localities (e.g., Livingstone Mountains, Mount Rungwe and Ngosi Crater) (see Table 5). It has recently been collected in Livingstone Mountains and on Mount Rungwe, but not near Ngosi Crater, despite recent, though limited, surveys. This latter point suggests that the populations might be experiencing decline, but further surveys are needed to fully understand the distribution, ecology, and possible population trends in these areas to inform future conservation strategies.

The populations of *Nectophrynoides viviparus* sensu stricto in the Southern Highlands are now considered restricted to the Southern Highlands with a much narrower range and smaller area of occurrence (see Table 5). The three new species described from the Udzungwa Mountains and Mahenge Mountains are also considered to have restricted distributions (Fig. 2). *Nectophrynoides luhomeroensis* is only found in high elevation forests and grassland mosaics that are under serious external pressures from both anthropogenic activities and climatic changes. However, *N. luhomeroensis* is relatively well protected as it is found inside the boundaries of the Udzungwa Mountains National Park, which provide some level of protection from encroachments. *Nectophrynoides uhehe* can be found in several locations across the Udzungwa Mountains landscape, however some of these forest fragments are impacted by anthropogenic activities, which could lead to local extinction of populations. There are a few known extinct populations of *N. uhehe* that occurred in fragments which no longer exist (for example, populations in Kiolela F.R.). This is of major concern for the remaining populations occurring in small, fragmented forest patches. *Nectophrynoides saliensis* can only be found in the very small forest fragment of Sali F.R., which is currently a forest reserve. The area is under serious threat of exploitation and deforestation, putting the survival of the species at risk, and demands conservation efforts for its long-term protection. Overall, the new concept of *N. viviparus*, and the newly described taxa, will have a more threatened conservation status given their limited distributions, and their highly threatened habitats with substantial changes following anthropogenic and climatic changes (IUCN 2025).

The description of three new species brings the number of known viviparous anurans to 20 species worldwide, of which 16 species belong to the genus *Nectophrynoides*. The distinctive reproduction and biology that these bufonids exhibit highlight the potential functional loss, if these species go extinct. The risk of losing these species and their contribution to functional diversity in amphibians should be reasons to increase conservation efforts in order to protect them. More surveys are needed to fully understand the behaviour and ecology of these remarkable arboreal viviparous bufonids.

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Supplementary Material 1

Files S1–S4

Authors: Thrane C, Lyakurwa JV, Liedtke HC, Menegon M, Petzold A, Loader SP, Scherz MD (2025)
Data type: .zip
Explanation notes: File S1. Morphometric data [.xslx file]. — **File S2.** Illustrations of measurements and key traits [.docx file]. — **File S3.** Museomic data [.xslx file]. — **File S4.** GenBank accession numbers [.xlsx file].

Link: https://doi.org/10.3897/vz.74.e167008.suppl1