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An integrative taxonomic revision and redefinition of *Gephyromantis* (*Laurentomantis*) *malagasius* based on archival DNA analysis reveals four new mantellid frog species from Madagascar

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Abstract

The subgenus *Laurentomantis* in the genus *Gephyromantis* contains some of the least known amphibian species of Madagascar. The six currently valid nominal species are rainforest frogs known from few individuals, hampering a full understanding of the species diversity of the clade. We assembled data on specimens collected during field surveys over the past 30 years and integrated analysis of mitochondrial and nuclear-encoded genes of 88 individuals, a comprehensive bioacoustic analysis, and morphological comparisons to delimit a minimum of nine species-level lineages in the subgenus. To clarify the identity of the species *Gephyromantis malagasius*, we applied a target-enrichment approach to a sample of the 110 year-old holotype of *Microphryne malagasia* Methuen and

Hewitt, 1913 to assign this specimen to a lineage based on a mitochondrial DNA barcode. The holotype clustered unambiguously with specimens previously named *G. ventrimaculatus*. Consequently we propose to consider *Trachymantis malagasia ventrimaculatus* Angel, 1935 as a junior synonym of *Gephyromantis malagasius*. Due to this redefinition of *G. malagasius*, no scientific name is available for any of the four deep lineages of frogs previously subsumed under this name, all characterized by red color ventrally on the hindlimbs. These are here formally named as *Gephyromantis fiharimpe* **sp. nov.**, *G. matsilo* **sp. nov.**, *G. oelkrugi* **sp. nov.**, and *G. portonae* **sp. nov.** The new species are distinguishable from each other by genetic divergences of >4% uncorrected pairwise distance in a fragment of the 16S rRNA marker and a combination of morphological and bioacoustic characters. *Gephyromantis fiharimpe* and *G. matsilo* occur, respectively, at mid-elevations and lower elevations along a wide stretch of Madagascar's eastern rainforest band, while *G. oelkrugi* and *G. portonae* appear to be more range-restricted in parts of Madagascar's North East and Northern Central East regions. Open taxonomic questions surround *G. horridus*, to which we here assign specimens from Montagne d'Ambre and the type locality Nosy Be; and *G. ranjomavo*, which contains genetically divergent populations from Marojejy, Tsaratanana, and Ampotsidy.

Keywords

Amphibia, Anura, archival DNA, Mantellidae, new species, phylogeography

Introduction

Among the hyperdiverse anuran fauna of Madagascar, with about 370 scientifically named species (Frost 2021; AmphibiaWeb 2021), many species are represented by only a few voucher specimens in collections. In some cases, this reflects the high proportion of microendemism in Madagascar's fauna (Wilmé et al. 2006; Vences et al. 2009) where species may occupy small ranges, often in remote mountain massifs that are extremely difficult to access. In other cases, species may be relatively widespread, but are difficult to detect, as has been shown for some fossorial taxa among reptiles (Brown et al. 2016). They may be characterized by secretive habits or may occur at low densities across their range.

One such secretive group of Madagascar frogs is the subgenus *Laurentomantis* in the genus *Gephyromantis*. These frogs have long been so poorly known that the monograph of Blommers-Schlösser and Blanc (1991) contained no information on their color in life, and the first photograph of a living specimen was published by Blommers-Schlösser and Blanc (1993). The six recognized nominal species of this subgenus form a clade (Kaffenberger et al. 2012) known from across Madagascar's eastern and northern rainforests, but the number of individuals known to science is very small for most species. For instance, *Gephyromantis horridus* (Boettger, 1880) was described based on a juvenile individual from the northern offshore island Nosy Be, but has never been collected there in recent times despite intensive surveys (e.g., Andreone et al. 2003); new collections include a few individuals from the Montagne d'Ambre and Tsaratanana massifs assigned to this species (Vences et al. 2002; Glaw and Vences 2007). *Gephyromantis ventrimaculatus* (Angel, 1935), originally described as *Trachymantis malagasia ventrimaculatus* from Isaka-Ivondro in extreme south-eastern Madagascar, is known from localities in the South East, Southern Central East and Northern Central East of Madagascar. It occurs in two of the most intensively studied rainforest sites in Madagascar, Ranomafana National Park and Analamazaotra-Mantadia National Park near Andasibe. Despite its conspicuous coloration with grey-bluish vermiculations on a black venter, only a few records of this species exist from Ranomafana and only a single specimen has ever been recorded from Andasibe (Vences et al. 2002; Glaw and Vences 2007; Randrianiaina et al. 2011; Strauß et al. 2013; Riemann et al. 2015). A further recently described species, *G. ranjomavo* Glaw and Vences, 2011, is known from the holotype collected in the Marojejy Massif in the North East and a second specimen from an unknown locality. Only *G. malagasius* (Methuen and Hewitt, 1913) and *G. striatus* (Vences, Glaw, Andreone, Jesu and Schimmenti, 2002) have been regularly reported from various sites in eastern or north-eastern Madagascar, respectively. However, as currently understood, *G. malagasius* (originally described as *Microphryne malagasia* Methuen and Hewitt, 1913), consists of several genetically deeply divergent lineages of uncertain taxonomic status (Vieites et al. 2009; Perl et al. 2014), and not all of them are commonly collected. Lastly, the recently described species *G. marokoroko* is known from only the type series collected at three nearby sites in the Northern Central East (Hutter et al. 2022).

Besides their apparent rareness, most species of the subgenus *Laurentomantis* are remarkable frogs by their very spiny and tubercular dorsal integument and have long been considered a separate genus (e.g., Blommers-Schlösser and Blanc 1991). These terrestrial frogs occur in the rainforest leaf litter, and calling males have been found on the ground or on moderately high perches in the vegetation, often at the bottom of slopes. The few known tadpoles in the subgenus (all of *G. ventrimaculatus*) were

non-feeding (Randrianiaina et al. 2011) and have been collected in small rainforest streams, although it is uncertain if they were accidentally washed into the streams from putative terrestrial nests. Some *Laurentomantis* are characterized by tibial glands of unknown function found in males and females; others have a ventral pattern of conspicuous bluish vermiculations on dark or reddish color primarily on the ventral sides of their hindlimbs. A more comprehensive understanding of the ecology of these frogs and the biological functions of their morphological and chromatic characteristics is hampered by the scarcity of field observations and a lack of understanding of their systematics.

The present study provides a comprehensive molecular assessment of the frogs in the subgenus *Laurentomantis*, based on DNA sequences of all samples collected over the past 20 years. To clarify the taxonomic status of lineages currently included in *G. malagasius*, we applied targeted enrichment sequencing to genetically characterize the 110-year-old holotype of this species, which unexpectedly was found to be conspecific with *G. ventrimaculatus*; this surprising result indicates that all recently collected frogs assigned to *G. malagasius* instead belong to four species new to science. We herein formally name and characterize these new species, based on an integration of molecular, bioacoustic and morphological information.

Materials and Methods

This study is based on voucher specimens and call recordings collected during various field campaigns in Madagascar between 1994–2017, and tissue samples collected since the year 2000. Upon collection in the field, frogs were anesthetized and euthanized by immersion in tricaine methanesulfonate (MS222) or chlorobutanol solution. We removed tissue samples for molecular analysis and stored them separately in 1.5 ml vials with 95% ethanol. Vouchers were then fixed in 95% ethanol (or in 12.5% formalin), preserved in 70% ethanol, and deposited at the Museo Regionale di Scienze Naturali, Torino (MRSN); the Zoologisches Forschungsmuseum A. Koenig, Bonn (ZFMK); Zoological Museum Amsterdam (ZMA; collection now included in Naturalis, Leiden); Zoologische Staatssammlung München (ZSM); and the Université d'Antananarivo, Mention Zoologie et Biodiversité Animale (UADBA). Additional material was studied from the Transvaal Museum Pretoria (TMP), the Museum National d'Histoire Naturelle, Paris (MNHN), Naturhistorisches Museum Wien (NMW), Naturhistorisches Museum Bern (NMBE), and Senckenberg Museum Frankfurt (SMF), and reference is made to material hosted at the Biodiversity Institute and Natural History Museum of the University of Kansas (KU). FGZC, FGMV and ZCMV refer to field numbers of F. Glaw and M. Vences. FAZC and FN refer to field numbers of F. Andreone. RJS, DLR, PSG, CRH, APR, JCR, MSZC and ACZCV refer to field numbers of Jasmin E. Randrianirina, Dina Ramamonjisoa, Philip-Sebastian Gehring, Carl R. Hutter, Achille P. Raselimanana, Jana Riemann, Mark D. Scherz and Angelica Crottini, respectively. Geographic regions within Madagascar are named according to Boumans et al. (2007).

The holotype of *Microphryne malagasia* Methuen and Hewitt, 1913, TMP 10076 (original number 'No. 1155'), was collected by Herschell-Chauvin in 1911 in a locality called Folohy (Methuen and Hewitt 1913b). The specimen is currently preserved in ca. 70% ethanol, but its precise preservation history is unknown.

Morphometric measurements were taken by MV with an accuracy of 0.1 millimeter with a manual caliper. The measurements and abbreviations used are: snout–vent length (SVL); maximum head width (HW); head length from tip of snout to posterior edge of mouth opening (HL); horizontal tympanum diameter (TD); horizontal eye diameter (ED); distance between anterior edge of eye and nostril (END); distance between nostril and tip of snout (NSD); distance between both nostrils (NND); forelimb length, from limb insertion to tip of longest finger (FORL); hand length, to the tip of the longest finger (HAL); hind limb length, from the cloaca to the tip of the longest toe (HIL); foot length (FOL); foot length including tarsus (FOTL); and tibia length (TIBL). We report webbing formula according to Blommers-Schlösser (1979) to ensure comparability with previous species descriptions of Malagasy frogs. Femoral gland terminology follows Glaw et al. (2000) and Vences et al. (2007).

We recorded vocalizations in the field using different types of tape recorders (Tensai RCR-3222, Sony WM-D6C) with external microphones (Sennheiser Me-80, Vivanco EM 238), digital recorders (Tascam DR05, Edirol R-09, Marantz PMD 661 MkII, Olympus LS-10, Zoom H5) with built-in microphones or external microphones attached (Sennheiser K6+ME-66, ME-67, MKH-8060), and in one case extracted sound files from a published audio CD (Rosa et al. 2011). Recordings were sampled or re-sampled at 22.05 kHz and 32-bit resolution and computer-analyzed using the software Cool Edit Pro 2.0. We obtained frequency information through Fast Fourier Transformation (FFT; width 1024 points) with Hanning window function. Spectrograms were drawn with Blackman window function at 256 bands resolution. In some cases sensitive filtering was applied to remove background sounds, applied only to frequencies outside the prevalent bandwidths of calls. Temporal measurements are summarized as range with mean ± standard deviation in parentheses and calls are described following note-centered scheme of Köhler et al. (2017). Calls have been deposited in the Macaulay Library (https://www.macaulaylibrary.org; reference numbers 274464–274474).

Two molecular dataset were assembled to examine the genetic variation and differentiation within the subgenus *Laurentomantis*:

(1) All available samples were DNA barcoded using a fragment of the mitochondrial 16S rRNA gene, which has previously been used as standard marker for Malagasy frogs (Vieites et al. 2009). DNA was salt-extracted and a

fragment at the 3′ terminus of the 16S rRNA gene amplified using the primer pair: 16SFrogL1/16SFrogH1 (5′–CATAATCACTTGTTCTTTAAA–3′;5′–GATCCAACATCGAGGTCG–3′) modified from Palumbi et al. (1991), and the following PCR protocol: initial denaturation for 90 s @ 94°C, followed by 36–40 cycles of denaturation for 45 s @ 94°C, primer annealing for 45 s @ 50–53°C and elongation for 90 s @ 72°C, followed by a final extension step for 5 min at 72°C.

(2) To understand the concordance between the variation in mitochondrial and nuclear-encoded genes, we amplified fragments of two nuclear-encoded genes: sacsin (SACS) and recombination-activating gene 1 (RAG1). For SACS, we applied the nested PCR approach of Shen et al. (2012) with primers SACSF2 (5′–AAYATHACNAAYGCNTGYTAYAA–3′) and SACSR2 (5′–GCRAARTGNCCRTTNACRTGRAA–3′) in the first round, and SACSNF2 (5′–TGYTAYAAYGAYTGYCCNTGGAT–3′) and SACSNR2 (5′–CKGTGRGGYTTYTTRTARTTRTG–3′) in the second round. PCR protocols for both rounds were identical, as suggested by Shen et al. (2012): 240 s @ 94°C, 45 × [45 s @ 94°C, 40 s @ 45°C, 120 s @ 72°C], 600 s @ 72°C. For RAG1, we used the primers Gephlut-RAG1-F1 (5′–ATGGAGAGCCAACCCCTATC–3′) and Gephlut-RAG1-R1 (5′–KCCAGACTCGTTTCCTTCRC–3′) (originally developed for a study of *Gephyromantis luteus*: Vences et al. 2021b) with the PCR protocol 120 s @ 94°C, 35 × [20 s @ 94°C, 50 s @ 53°C, 180 s @ 72°C], 600 s @ 72°C.

We purified PCR products with Exonuclease I and Shrimp Alkaline Phosphatase digestion, and the purified products along with sequencing primers were shipped to LGC Genomics (Berlin) for sequencing on automated capillary sequencing instruments. The 16S fragment was sequenced with the forward PCR primer only, SACS and RAG1 were sequenced with the PCR primers in both directions and the two strands combined. Chromatograms were checked for base-calling errors and edited with CodonCode Aligner 6.0.2 (Codon Code Corporation, Dedham, MA, USA) and newly determined sequences submitted to GenBank (accession numbers OM885271–OM885340 and OM897144–OM897211).

To obtain DNA sequences from the holotype of *Gephyromantis malagasius*, we applied targeted enrichment sequencing (Straube et al. 2021) following a 'barcode fishing' strategy that we have previously employed and described in previous studies with Malagasy frogs (Rancilhac et al. 2020; Scherz et al. 2020). This approach aims at obtaining sequences of the same 16S rRNA gene fragment that we also PCR-amplified and sequenced from the fresh samples. The strategy uses targeted enrichment with 5,962 baits of 70 nucleotides in length, after filtering based on melting temperature and collapsing 99% identical baits. The baits were designed by Arbor Biosciences using sequences from most Malagasy frog species, including various *Gephyromantis* species of the subgenus *Laurentomantis*. A thigh muscle tissue sample was extracted from the type of *G. malagasius* using DNA-free scissors and stored in 100% ethanol in a 1.5 ml tube filled in a lab naïve to *Gephyromantis* research. We then performed DNA extraction in a clean lab dedicated to museum specimen and ancient DNA analysis where no other sample of the subgenus *Laurentomantis* had been processed before. The sample was washed with a GuSCN-based Qiagen PE Buffer, DNA extracted following the protocol of Rohland et al. (2004), and purification following the protocol of Dabney et al. (2013). The GuSCN-based extraction buffer from Rohland et al. (2004) has been widely shown to be effective in releasing DNA from museum specimens, while the silica column-based purification from Dabney et al. (2013) is especially suitable for retaining short fragments. This combination was also found to perform best with wet-collection specimens in an extensive comparison of different approaches (Straube et al. 2021). Specifically, tissue samples were extracted with an Guanidine thiocyanate extraction buffer (5 M GuSCN, 50 mM Tris pH 8.0, 25mM NaCl, 20 mM EDTA, 1% Tween-20, 1% 2-mercaptoethanol) as described in Rohland at al. (2004). Samples were incubated for 18 hours rotating at room temperature. The supernatant was then added to 13 mL binding buffer (5 M guanidine hydrochloride, 40% Isopropanol, 0.05% Tween-20, 90 mM sodium acetate) as described in Dabney at al. (2013). DNA was purified using the MinElute silica spin columns (Qiagen).

For library preparation, we used a single-stranded (ssDNA) approach optimized for ancient and archival DNA (Gansauge et al. 2013, 2017) with custom adapters from Gansauge et al. (2013), amplified with custom Illumina indexing primers described in Paijmans et al. (2017) after determining the optimal cycle number using qPCR (Gansauge et al. 2013; Basler et al. 2017).

The ss-DNA library of the *G. malagasius* holotype was then captured twice for the aforementioned target sequences using the Arbor Biosciences MyBaits kit (RNA-based in-solution sequence capture), with 14.5 µL of each indexed library in a 24 h reaction at a hybridisation temperature of 65°C, and following the MyBaits target enrichment protocol except reducing the bait volume to 2.75 µL and substituting the missing 2.75 µL in each reaction with nuclease-free water. After hybridization, the libraries were bound to streptavidin-coated magnetic beads, and the reactions washed and eluted according to the MyBaits kit protocol. We then performed PCR amplification in a reaction volume of 60 µL with the following PCR conditions: 120 s @ 95°C, then with an optimal cycle number determined using qPCR, 30 s@ 95°C, 45 s @ 60°C, 45 s @ 72°C, and final extension of 180 s @ 72°C. Amplifications were purified using a Min Elute PCR Purification Kit (Qiagen), with final elution in a total volume of 30 µL of 10 nM Tris-HCl, 0.05% TWEEN-20 solution (pH 8.0). This procedure was performed twice to increase target capture reactions success, as described in Li et al. (2015) and Paijmans et al. (2016). Qubit 2.0 and 2200 TapeStation (Aligent Technologies) assays were used to determine the final library concentration and length distribution. We sequenced the enriched library on an Illumina Next-Seq 500 sequencing platform using 500/550 High Output v2.5 (75 cycles SE, aimed at 3 million reads) with custom sequencing primers (Paijmans et al. 2017).

After quality-trimming and adapter removal, all reads (duplicates not removed to keep information of read frequency) were compared against reference sequences of various *Laurentomantis* species using a custom script described in Rancilhac et al. (2020), with a similarity threshold to the references of 90%, to reduce the data set for further analysis. The selected reads were then uploaded in CodonCode Aligner 6.0.2 (CodonCode Corp.) and three majority-based alignments performed to align reads to alternative reference sequences of *Laurentomantis* species (corresponding to *G.* "*ventrimaculatus*" sensu lato, *G.* "*malagasius*" lineage B, and *G. striatus*). For this purpose, we used the "Align to Reference" option building a majority rule consensus, discarding stretches with <50 reads, filling uncovered regions of the reference sequence with N, with a local alignment approach (minimum percent identity = 70%, word length = 8, match score = 1, mismatch penalty = –2, gap penalty = –2, additional first-gap penalty = –3, minimum overlap score = 40). The contigs resulting from the three individual alignment attempts were identical. The respective sequence was deposited in GenBank (accession number OM897120) and analyzed along with the set of 16S DNA sequences of fresh samples.

We aligned the sequences for each locus individually in MEGA7 (Kumar et al. 2016) with the Muscle alignment option. As the alignment was unambiguous and only required few single indels for 16S (especially in the outgroup), all sites were used for phylogenetic analysis. All alignments are available from Figshare, DOI: https://doi.org/10.6084/m9.figshare.19299884.

The 16S alignment was analyzed with a relatively simple (K2P) substitution model to avoid overparametrization for shallow branches, in a Maximum Likelihood analysis in MEGA 7 with NNI branch swapping, and 500 nonparametric bootstrap replicates to assess node support. We calculated uncorrected pairwise distances between 16S sequences using the program TaxI2, implemented in iTaxoTools (Vences et al. 2021a). For this purpose, we used a reduced set of 47 sequences that spanned a full length of 508 nucleotides, thus avoiding biases that could arise when excessively short sequences (consisting mainly of the hypervariable central stretch of the 16S fragment) are included (reduced alignment available from Figshare: DOI: https://doi.org/10.6084/m9.figshare.19299884).

The two nuclear-encoded genes (RAG1 and SACS) were analyzed separately from the mitochondrial gene and each other since our main interest was to understand concordance (or absence thereof) in the differentiation of unlinked genetic markers. We used a haplotype network visualization to graphically represent the relationship among alleles (haplotypes) of these genes. Haplotypes were estimated with the PHASE algorithm (Stephens et al. 2001) implemented in DnaSP (Version 5.10.3; Librado and Rozas 2009) with default parameters. The phased sequences were used to reconstruct Maximum Likelihood trees with the Jukes-Cantor substitution model in MEGA 7 (the simplest available model, to avoid overparametrization), and these were used as input for Haploviewer (written by G. B. Ewing; http://www.cibiv.at/~greg/haploviewer), a software that implements the methodological approach of Salzburger et al. (2011).

As in previous studies, we follow the general lineage concept (de Queiroz 1998, 2007) in combination with a relaxed biological species criterion, i.e., demanding reproductive isolation indicated by restricted gene flow among lineages (e.g., Speybroeck et al. 2020). Because reproductive barriers generated through time increase genealogical depth and agreement among unlinked loci (Avise and Wollenberg 1997), we use genealogical concordance (Avise and Ball 1990) between mitochondrial and nuclear loci, especially in populations occurring in sympatry or close geographical proximity, as an indicator for restricted gene flow. We then assigned species status to a lineage, along with concordance between genetic and morphological evidence (Padial et al. 2010).

Results

Molecular phylogenetics and genetic divergence

The phylogenetic analysis of the 16S sequences of 88 individuals of *Laurentomantis* (plus one sequence of *Gephyromantis* (*Vatomantis*) *webbi* used as outgroup; total alignment length 508 nucleotides) revealed nine major clades separated by uncorrected pairwise distances >4%, which we consider as candidate species-level lineages (Fig. 1). Some of these contain intra-lineage variation of up to 5%; these cases will be discussed below. All of the nine lineages are supported by bootstrap values of at least 90%, whereas nodes defining inter-lineage relationships received only in one case a support of 70%, and 63% or less for all other nodes. Our tree does not constitute a reliable hypothesis of relationships among the main lineages, but provides a reliable assignment of individuals into lineages.

The archival DNA analysis of the holotype of *Microphryne malagasia* yielded 3,481,149 raw sequence reads, of which 11,118 reads (0.32%) were retained after the filtering pipeline. These were used for downstream analysis for the sample, which led to a consensus sequence built with 10,497 reads. In the phylogenetic analysis, this consensus sequence clustered among specimens of the lineage commonly considered *G. ventrimaculatus* (e.g., Vences et al. 2002; Glaw and Vences 2007; Vieites et al. 2009; Perl et al. 2014). This clustering is supported by a bootstrap value of 94%. This assignment of the *malagasius* type is supported by diagnostic SNPs in different parts of the 16S fragment (Supplementary Fig. S1); this confirms that the phylogenetic assignment of the consensus sequence is due to substitutions in various sequence fragments obtained separately by the capture approach.

The molecular evidence suggests that the name *G. malagasius* should take nomenclatural priority to refer to those frogs currently considered as *G. ventrimacula-*

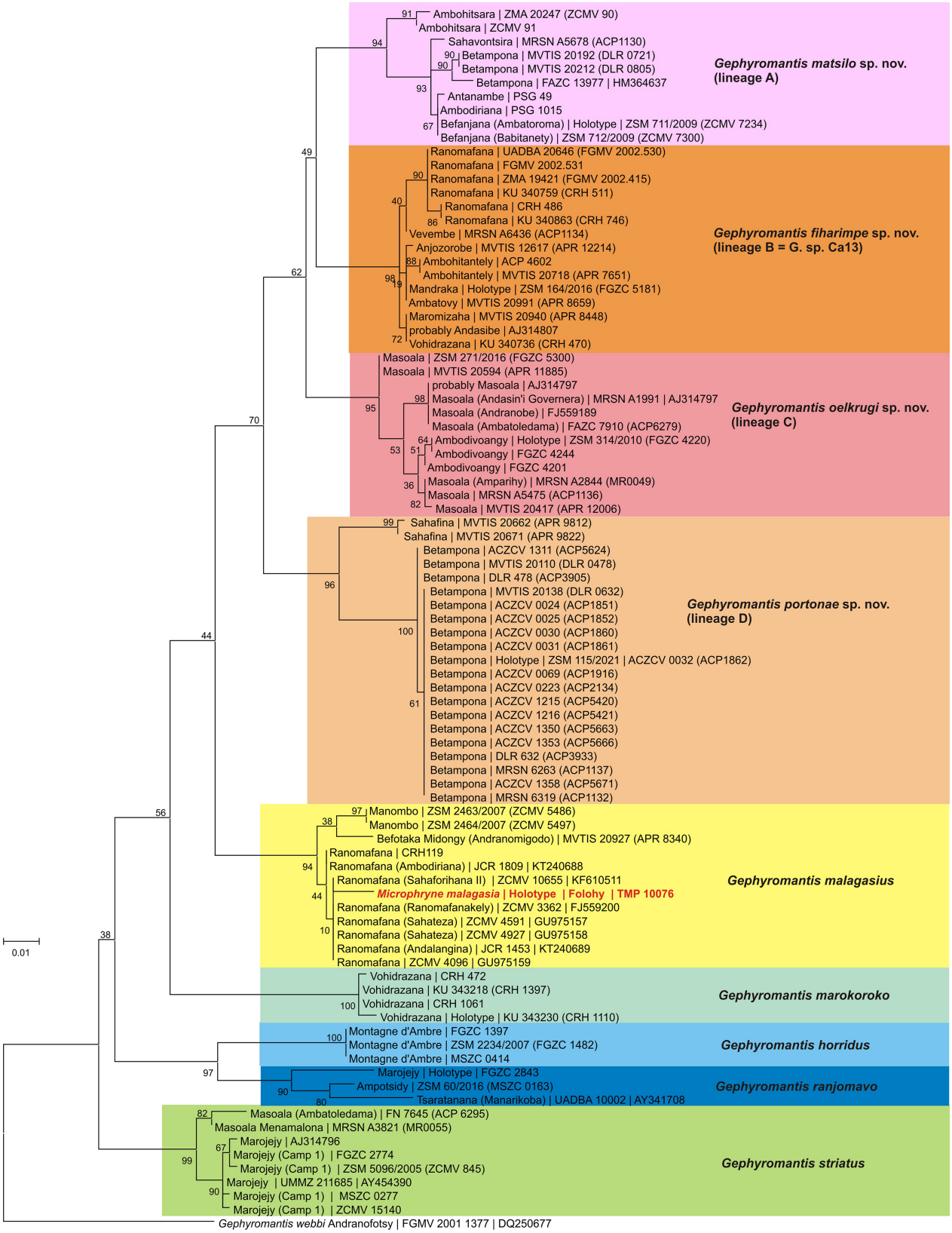

Figure 1. Maximum Likelihood phylogenetic tree inferred from an alignment of 508 nucleotides of the mitochondrial 16S rRNA gene, in 88 individuals of *Gephyromantis* belonging to the subgenus *Laurentomantis*. A species of the subgenus *Vatomantis* (*G. webbi*) was used as the outgroup. Numbers at nodes are bootstrap values in percent (500 replicates; not shown if <50%). Colors correspond to main species-level lineages as discussed in the text.

tus. In order to assess whether the name *Trachymantis malagasia ventrimaculatus* Angel, 1935 represents a junior synonym of *Microphryne malagasia* Methuen and Hewitt, 1913, a comparison of the respective name-bearing types is necessary: (1) The lectotype of *Trachymantis malagasia ventrimaculatus*, examined previously by

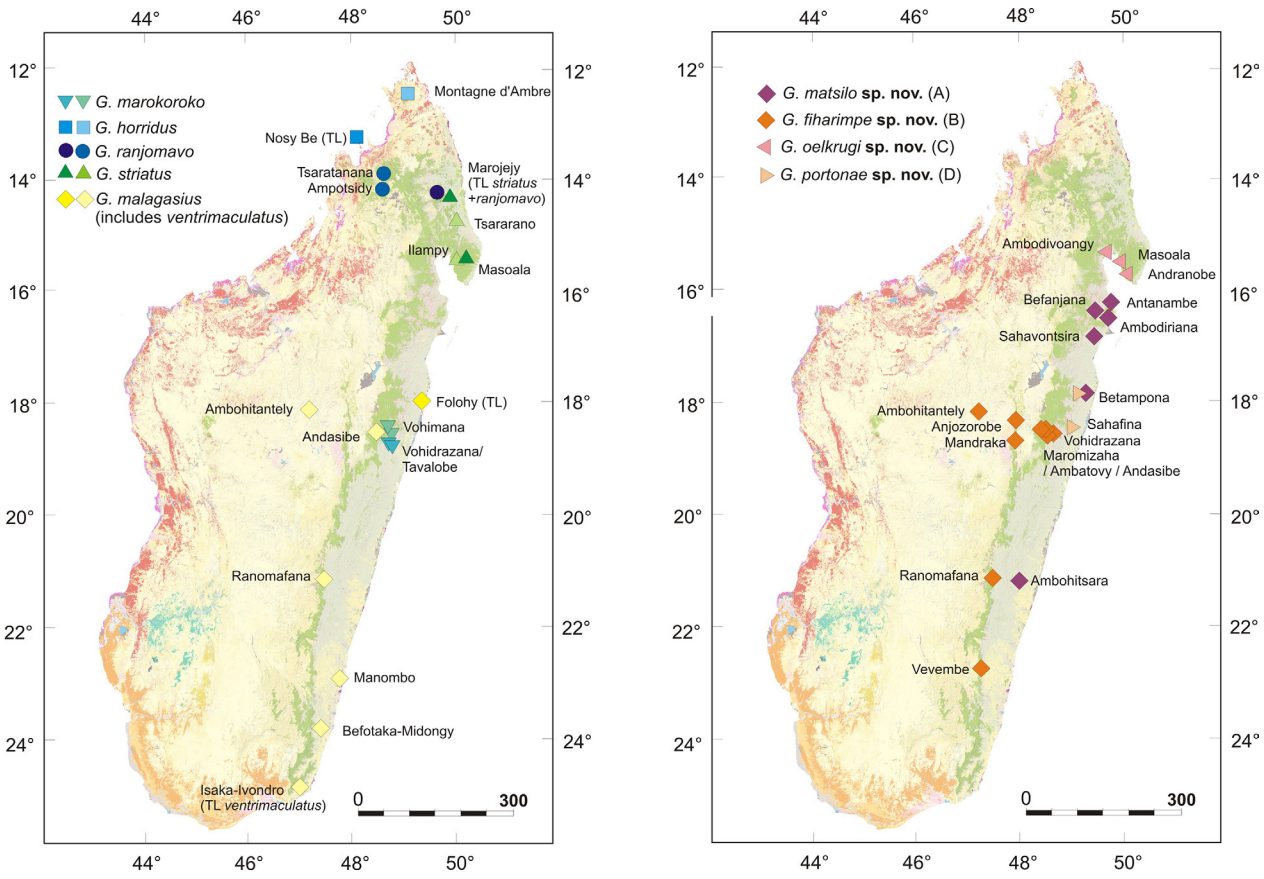

Figure 2. Maps showing locality records in Madagascar considered in this study, the majority of them based on molecular data. The left map shows records for *G. marokoroko*, *G. horridus, G. malagasius, G. ranjamavo,* and *G. striatus* in paler colors if they would benefit from further confirmation or taxonomic revision, as follows: pale yellow marks the type locality of *G. ventrimaculatus* (specimens not studied genetically) and records based on individuals phenotypically matching the types of *ventrimaculatus* (here considered to be a junior synonym of *G. malagasius*). Light green marks records assigned to *G. striatus* based on phenotype without genetic data. Light blue marks a record assigned to *G. horridus* tentatively due to the lack of genetic data from the type locality. Sky blue mark genetically divergent specimens here assigned to *G. ranjomavo* but in need of further study. The type locality of *G. malagasius,* Folohy, is here tentatively placed close to Toamasina, but its exact location is uncertain (north of Toamasina / east of Lake Alaotra, see species account).

Vences et al. (2002), shares with other individuals previously referred to *G. ventrimaculatus*, a highly contrasted ventral pattern of light, more or less vermiculated spots and markings on dark background, which in this expression is unique within the subgenus *Laurentomantis*. Since this color pattern allows for an unambiguous definition of *G. ventrimaculatus* sensu lato, we did not attempt to sequence the *ventrimaculatus* lectotype. (2) The holotype of *Microphryne malagasia* lacks this typical contrasted ventral pattern; the color of this specimen was almost completely faded upon examination in 2021, and ventral vermiculation also was not mentioned in the original description (Methuen and Hewitt 1913b). It is however worth noting that the description also lacks a mention of reddish ventral coloration, the main pattern characterizing all those lineages commonly assigned in the past to *Gephyromantis malagasius*. We therefore suspect that already in 1913, two years after its collection in Folohy, the color of the specimen was largely faded. Hence, we here anticipate our taxonomic conclusions below, and redefine *G. malagasius* as the lineage containing frogs with the typical ventral pattern of *ventrimaculatus;* this implies *G. ventrimaculatus* becoming a subjective junior synonym of *G. malagasius*.

As a consequence, no scientific name is available for those lineages that include the frogs with red ventral color on limbs that were assigned to *Gephyromantis malagasius* by Blommers-Schlösser and Blanc (1993), Vences et al. (2002), Glaw and Vences (2007) and other authors. The newly defined *G. malagasius* is separated from the other eight *Laurentomantis* species-level lineages by uncorrected pairwise distances in the 16S rRNA gene of 6.4–9.1%. Intra-lineage distances reach 2.9% between specimens from Befotaka and Ranomafana.

The red-legged individuals traditionally assigned to *G. malagasius* are placed by our analysis into four deep mitochondrial lineages, in this section provisionally named A–D. Lineage A (bootstrap support BS=94%) occurs at mainly low-elevation sites in Madagascar's Southern and Northern Central East, from Ambohitsara (close to Ranomafana National Park) to Betampona and Befanjana Forest. Lineage B (BS=98%) occupies roughly the same region but appears to occur at mid-elevations, ranging from Ranomafana National Park to the Andasibe region

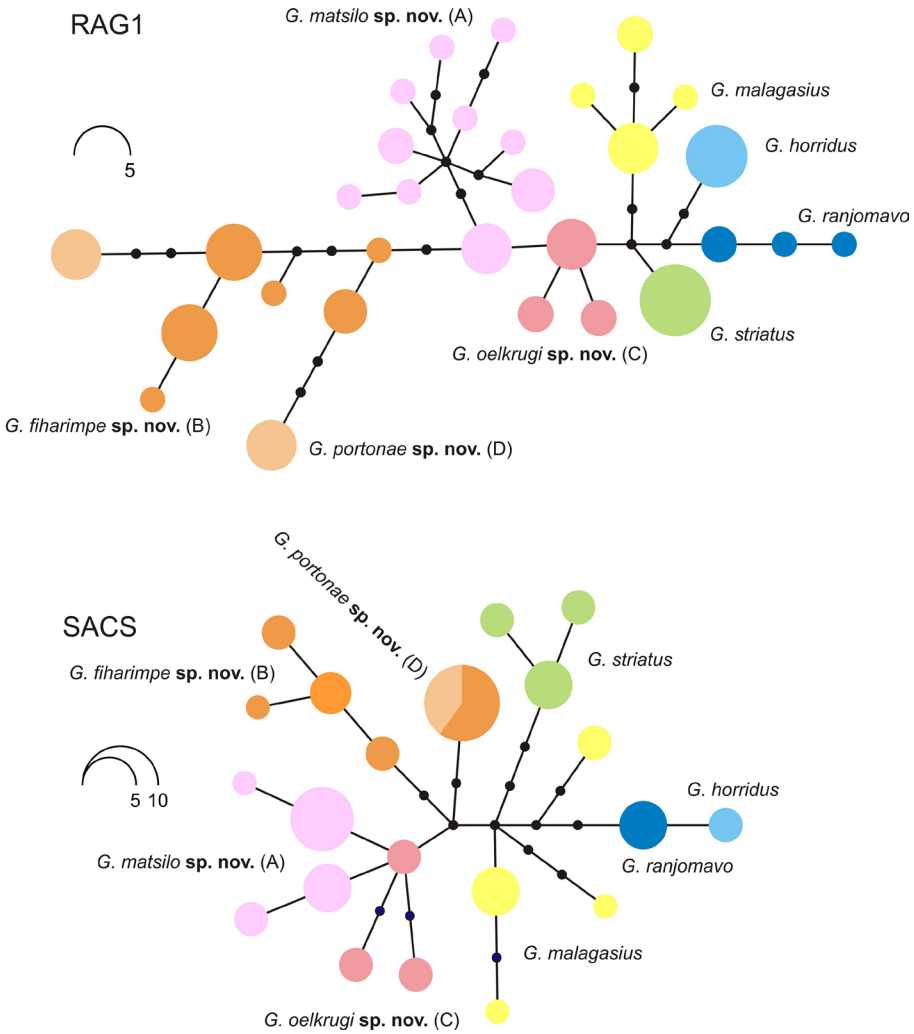

Figure 3. Haplotype networks based on sequences of the nuclear-encoded genes RAG1 (475 nucleotides; 74 phased sequences corresponding to 37 individuals) and SACS (834 nucleotides; 60 phased sequences corresponding to 30 individuals). Colors correspond to sequences of individuals assigned to lineages based on mitochondrial DNA (Fig. 1). Sequences were phased into haplotypes before analysis, and each individual is therefore represented with two sequences in each network.

and Anjozorobe. Lineage C (BS=95%) is only known from low elevations at the north-eastern localities Masoala and Makira. And finally, lineage D (BS=96%) is known from the Northern Central East, specifically from Sahafina and Betampona. These four lineages form a clade in our tree, which however did not receive strong bootstrap support. The smallest 16S uncorrected pairwise distances of 4.0–5.7% are found between lineages A and B, whereas the other comparisons among these four lineages yielded distances of 4.3–8.1%. Intra-lineage distances are up to 3% in lineage A, 1.8% in lineage B, 1.4% in lineage C, and 3.9% in lineage D.

Two further lineages correspond to the nominal species *G. marokoroko* and *G. striatus* and are confirmed by the analysis, with 100% and 99% bootstrap support, respectively. For *G. marokoroko* only individuals from the type locality (Vohidrazana) were available, and intra-lineage divergences are therefore negligible (<1%).

The final two species-level lineages revealed by our 16S tree correspond to a complex of enigmatic and poorly known taxa from northern Madagascar. They comprise specimens from Manarikoba forest in the Tsaratanana Massif that previously were assigned to *G. horridus* (Vences et al. 2002); however, in our tree, the single sequence obtained from these specimens clusters with high support (90%) with the holotype of *G. ranjomavo*, and with a specimen from Ampotsidy that phenotypically is very similar to *G. ranjomavo*. The three sequences substantially differ from each other (pairwise 16S distances 3.0–4.9%) but we here subsume all three under *G. ranjomavo* preliminarily. The second lineage contains individuals from Montagne d'Ambre that phenotypically resemble *G. horridus* from its type locality Nosy Be, and that we assign to this species preliminarily. The *G. horridus* + *G. ranjomavo* clade is supported by a bootstrap value of 97%, and the two species are separated by 6.7–8.3% 16S divergence.

Analysis of the nuclear-encoded genes RAG1 (alignment length 475 nucleotides for 37 samples) and SACS (834 nucleotides for 30 samples) suggested genealogical concordance across unlinked markers in the differentiation of most lineages described in the previous paragraphs (no sequences of these two genes were available for *G. morokoroko*). The haplotype networks of both genes (Fig.

3) show a relatively limited amount of variation, with a maximum of 14 mutational steps in RAG1 and nine mutational steps in SACS, but without haplotype sharing between lineages except for lineages B and D which had intertwined haplotypes (without sharing) in RAG1, and had one shared haplotype in SACS.

Morphological differentiation

In this study we limit our morphological comparisons to those lineages for which we provide relevant novel taxonomic information compared to Vences et al. (2002) and Glaw and Vences (2011), namely *G. horridus*, *G. ranjomavo*, *G. ventrimaculatus* sensu lato, and lineages A–D. For additional information on the remaining taxa, see Vences et al. (2002).

The molecular data suggest a need to re-assess the identity of specimens from the Manarikoba Forest in the Tsaratanana Massif previously assigned to *Gephyromantis horridus* (Vences et al. 2002). Our tree suggests that the single specimen sequenced from Manarikoba is genetically close to an individual from Ampotsidy, and together they form the sister group of the holotype of *G. ranjomavo*. The Ampotsidy specimen, in life, shows a color pattern reminiscent of *G. ranjomavo*, with reddish brown dorsal color on hindlimbs and forelimbs (yellowish brown in the *G. ranjomavo* holotype), differing from the predominantly blackish and brown dorsum (Fig. 7). A further character shared by the male specimen from Ampotsidy with the known males from Manarikoba and the male type specimen of *G. ranjomavo* is the presence of a distinct tibial gland (Vences et al. 2002; Glaw and Vences 2011).

No photographs of the Manarikoba specimens in life were available, but we assign this population tentatively to *G. ranjomavo* based on the genetic similarity to the Ampotsidy individual and to the *G. ranjomavo* holotype. The two specimens of *G. horridus* from Nosy Be, from historical collections of the 19th century (Fig. 4), do not show a tendency of lighter color dorsally on the limbs, and instead have both a relatively distinct pattern of two dark brown / blackish markings on the dorsum, at the area between the forelimbs, and at the posterior dorsum. These markings are also visible in life in specimens from Montagne d'Ambre, where in the single known male individual (Fig. 5) they take the form of two distinct transverse bands. This supports assigning the Montagne d'Ambre population to *G. horridus*, pending new collections and molecular data from the type locality. The single known male from Montagne d'Ambre (Fig. 5) has no tibial glands, which allows a distinction from all males attributed to *G. ranjomavo* (see above). From Nosy Be, unfortunately, no males have been collected and this character therefore remains unverified for topotypical *G. horridus*.

The synonymy of *G. ventrimaculatus* with *G. malagasius* as supported by the genetic data from the holotype of *G. malagasius* is surprising. However, examination of the holotype of *malagasius* (Fig. 14) revealed it is characterized by a remarkably tubercular dorsum, with large warts, several taking the form of short prominent ridges. Such a tubercular integument is also typical for the lectotype of *G. ventrimaculatus* (Fig. 14) and for individuals previously assigned to this species. Among the lineages that in the recent past have been assigned to *G. malagasius*, three occur near the *malagasius* type locality Folohy: lineages A, B and D. Of these, lineages A and B usually have a much less tubercular dorsum, whereas in lineage D the dorsum is also strongly tubercular.

Individuals of lineages A, B, C and D (all previously assigned to *G. malagasius*) are characterized by reddish color on the posteriormost portion of the belly and the ventral side of the thighs (Figs. 17, 20, 22, 24). The reddish color partly extends on the ventral surface of shanks, covers a part of the inguinal region, and often also forms a small patch at the insertion of forelimbs. The reddish color appears more intense in lineage D than in lineage B. Males of lineage B can easily be distinguished from those of lineages A, C and D by the presence of a distinct tibial gland (lacking in the other three lineages). Individuals of lineage C are characterized by their head and dorsum being covered by prominent and distinct, partly spine-like tubercles (Fig. 22), which are less prominent in specimens of lineages A and B and coarser and less spiny in lineage D. Individuals of lineage D have the third toe distinctly longer than the fifth.

Bioacoustic differentiation

Advertisement calls in the subgenus *Laurentomantis* are multi-note calls, containing non-tonal notes. Calls are usually emitted in call series. The calls studied herein are all somewhat similar and simple in their structure, namely pulsatile or pulsed notes repeated at regular intervals, with only one exception where note repetition within calls is rather irregular. Despite the overall similar structure of advertisement calls in this species complex, detailed comparison of call parameters among the genetically identified clades reveals more or less pronounced differences in certain characters.

Among all calls analyzed, calls assignable to *G. ranjomavo* are longest in duration (1780–2526 ms) and differ from calls of *G. horridus* (call duration 543–618 ms) by notes consisting of a single pulse only (versus 2–4 pulses/note), low note repetition rate within calls (10.8–14.2 vs. 29.4–31.0 notes/second), and higher dominant frequency (2348–3204 vs. 1172–1369 Hz).

Calls of *G. striatus* contain very short single-pulse notes, but mainly differ from calls of *G. ranjomavo* by shorter call duration (439–1360 vs. 1780–2526 ms) and higher note repetition rate within calls (25.6–33.5 vs. 10.8–14.2 notes/second).

Calls of the recently described *G. marokoroko* contain multi-pulsed notes with clearly separated pulses. They are similar to calls of *G. horridus* in general character, but mainly differ by lower note repetition rate (18.2–19.6 vs. 29.4–31.0 notes/second) and higher dominant frequency (2916–3192 vs. 1172–1369 Hz).

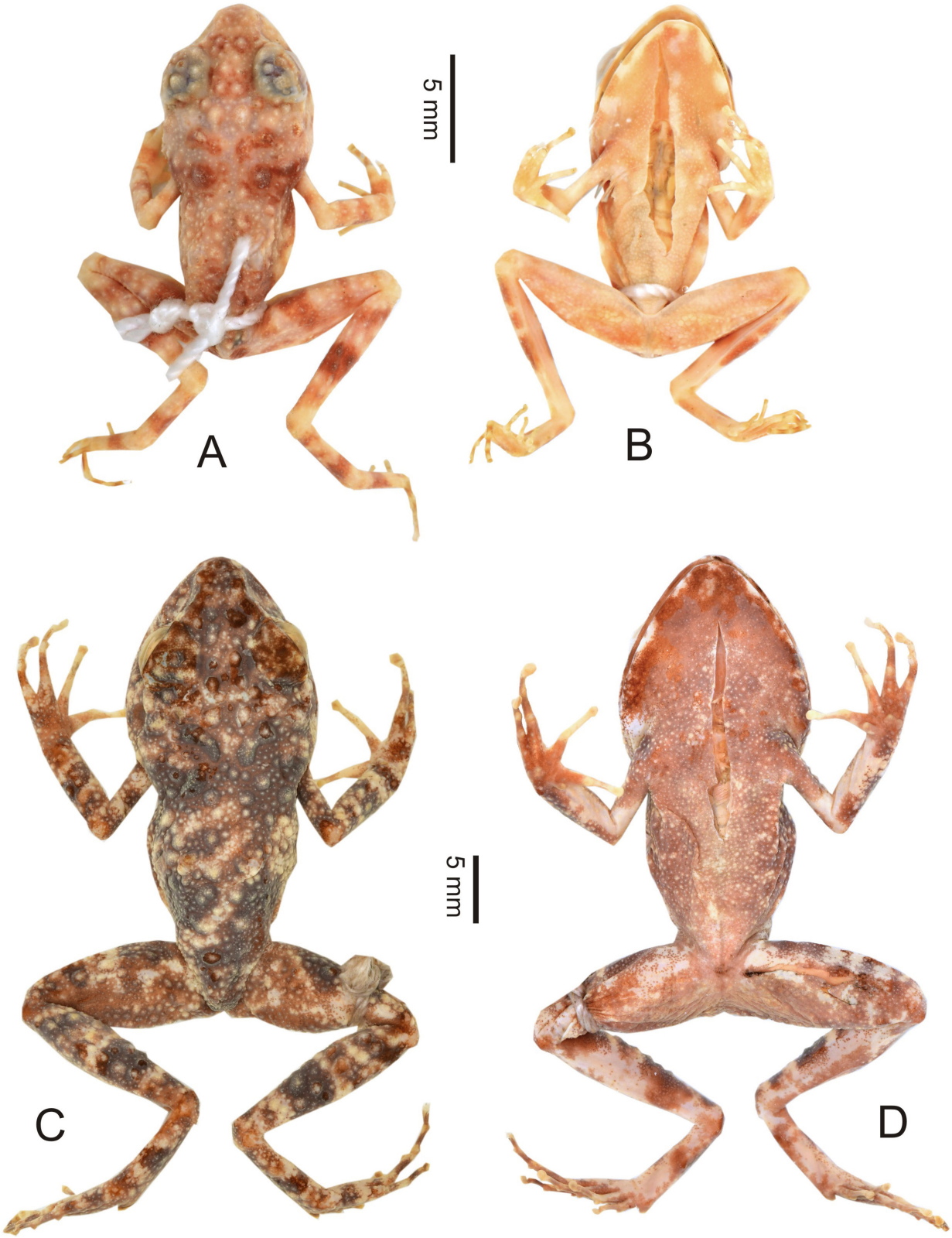

Figure 4. Preserved specimens of *Gephyromantis horridus* from the type locality Nosy Be. **A**, **B** Holotype SMF 7177 (juvenile) in dorsal and ventral views. **C**, **D** Specimen NMW 3643 (female) in dorsal and ventral views.

Calls assignable to *G. malagasius* (previously reported under its synonym *ventrimaculatus*) contain multi-pulsed notes, with 2–10 pulses being partly fused and thus not always clearly distinguishable in oscillograms. Interestingly, calls from Vohiparara and Manombo generally agree in call duration, note duration and note structure, but differ considerably by a doubling number of notes per call and consequently much higher note repetition rate within calls of the Manombo population, which together with the sample from Befotaka Midongy forms a subclade within *G. malagasius*.

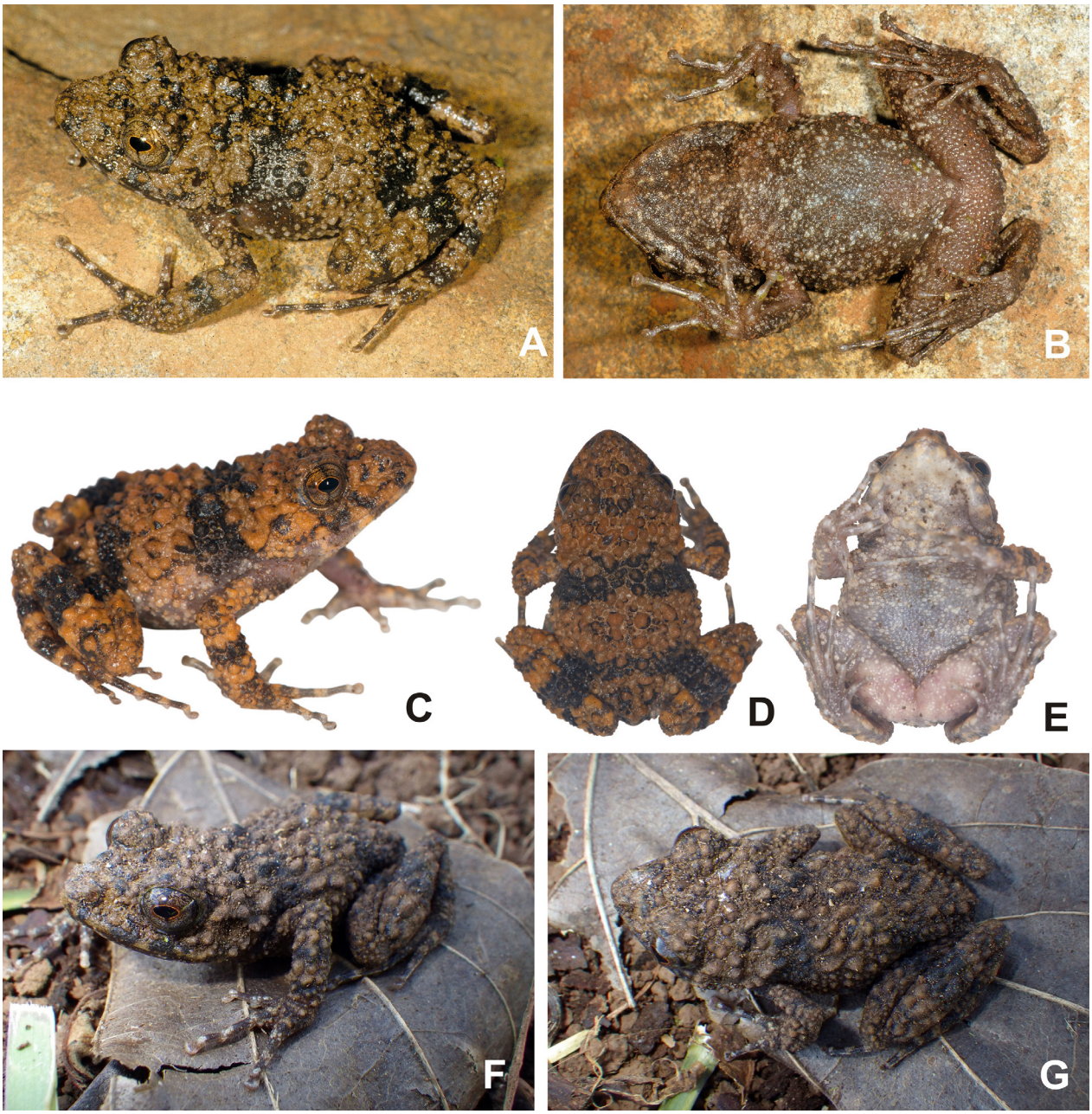

Figure 5. Specimens assigned to *Gephyromantis horridus* from Montagne d'Ambre National Park in life. **A**, **B** Adult female photographed in March 1994 (ZFMK 57433) in dorsolateral and ventral views. **C**–**E** Adult male (ZSM 126/2018) in dorsolateral, dorsal and ventral views. **F**, **G** Adult female discovered under rotten wood in May 2014 (not collected) in dorsolateral and dorsal views. Note the absence of tibial glands in all specimens. Not to scale.

Among the calls assignable to the four unnamed lineages identified in the *G. malagasius* complex, calls of lineages A and C both contain multi-pulsed notes with a clear pulse structure, with the calls of lineage A containing a higher number of pulses per note (6–10 vs. 1–4) and exhibiting a lower note repetition rate within calls (20.8–21.6 vs. 43.5–54.1 notes/second) when compared to lineage C. Calls of lineage B from Andasibe, Ankeniheny and Vohidrazana contain single-pulse notes only and are unique among all calls analyzed in exhibiting rather variable note repetition rate within calls. Calls of lineage B from Ambatolahy (Ranomafana National Park) are generally in agreement with the latter, but differ slightly by exhibiting a regular note repetition within calls and more pronounced amplidute modulation. No call recordings are available from lineage D. For detailed call descriptions and comparison see species accounts and Table 2.

Taxonomy

Our results suggest the necessity of taxonomic changes in the subgenus *Laurentomantis*. We re-define *G. malagasius* by considering *Trachymantis malagasia ventrimaculatus* Angel, 1935 as a junior synonym of *Microphryne malagasia* Methuen and Hewitt, 1913. This leaves four

Table 1. Morphometric measurements (all in mm) of voucher specimens of *Gephyromantis* belonging to the subgenus *Laurentomantis* newly examined for this study and identified on the basis of DNA sequence data. See Vences et al. (2002) and Glaw and Vences (2011) for measurements of additional individuals. For abbreviations of measurements, see Materials and Methods. HT, holotype.

Voucher	Field number	Status	Sex	Locality	SVL	HW	HL	ED	HTD	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIBL	FGL	FGW
G. horridus																				
ZSM 126/2018	MSTEMP 1		M	Montagne d'Ambre	33.5	13.2	13.8	4.8	2.3	3.7	2.3	3.5	24.3	11.5	53.6	23.8	15.6	15.8	7.7	4.8
G. ranjomavo																				
ZSM 60/2016	MSZC 0163		M	Ampotsidy	26.6	10.5	10.6	4.0	2.2	2.8	2.0	2.8	19.0	9.2	41.9	18.6	11.7	12.6	3.1	2.6
G. malagasius (=*ventrimaculatus*)																				
ZSM 2464/2007	ZCMV 5497		M	Manombo	27.0	10.7	11.4	4.4	1.8	3.3	1.8	2.2	19.0	8.6	44.1	18.9	12.0	14.0	5.8	2.4
ZSM 2463/2007	ZCMV 5486		F	Manombo	29.8	11.7	13.0	4.3	2.0	3.2	2.1	2.9	24.7	11.4	51.4	22.8	14.5	16.9	NA	NA
ZSM 537/2006	ZCMV 3362		M	Ranomafana	23.6	9.9	10.0	3.7	2.0	3.2	1.7	2.4	19.0	8.8	40.8	17.2	11.2	12.7	5.4	2.0
G. matsilo **sp. nov.** (lineage A)																				
ZSM 711/2009	ZCMV 7234	HT	M	Ambatoroma, Befanjana	21.9	8.8	8.9	3.4	1.7	2.7	2.0	2.2	17.0	8.0	37.1	16.1	10.8	12.2	5.1	2.0
ZSM 712/2009	ZCMV 7300	PT	F	Babitanety, Befanjana	24.9	9.6	10.9	4.0	2.0	2.8	1.8	2.6	18.6	8.5	42.1	17.8	11.1	13.1	NA	NA
ZMA 20247	ZCMV 90	PT	M	Ambohitsara	21.7	8.9	9.3	3.5	1.7	2.7	1.2	2.1	18.1	8.8	39.7	17.5	11.4	11.9	5.8	2.3
G. fiharimpe **sp. nov.** (lineage B)																				
ZSM 164/2016	FGZC 5181	HT	M	Mandraka	23.4	8.9	9.6	3.2	1.7	3.2	2.0	2.4	17.5	8.1	41.0	18.4	12.2	13.0	3.7	1.6
ZMA 19421	FGMV 2002.415	PT	F?	Ranomafana	25.8	9.2	10.7	3.4	1.9	2.7	1.7	2.0	18.5	9.2	43.5	19.3	12.5	13.3	NA	NA
G. oelkrugi **sp. nov.** (lineage C)																				
ZSM 271/2016	FGZC 5300	PT	F?	Masoala	21.1	8.4	8.7	3.3	1.8	2.8	1.5	2.3	14.8	7.1	32.6	14.5	9.6	10.1	NA	NA
ZSM 315/2010	NA	PT	M	Ambodivoangy	21.6	8.7	8.9	3.7	2.2	2.1	1.2	2.6	16.9	17.8	38.9	16.7	11.0	12.0	3.9	1.6
ZSM 314/2010	FGZC 4220	HT	M	Ambodivoangy	21.9	8.4	9.0	3.0	2.3	3.1	1.2	2.4	15.0	6.8	33.8	14.8	9.9	11.0	3.2	2.0
G. portonae **sp. nov.** (lineage D)																				
ZSM 115/2021	ACZCV 0032	HT	M	Betampona	22.9	9.0	10.7	3.4	1.7	2.9	1.6	2.7	17.0	8.2	36.1	16.1	10.5	11.5	5.5	2.5

Voucher	Field number	Status	Sex	Locality	SVL	HW	HL	ED	HTD	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIBL	FGL	FGW
ZSM 118/2021	ACZCV 0223	PT	F	Betampona	26.1	9.8	10.5	4.0	1.9	3.0	1.3	2.8	19.3	9.5	45.2	20.3	12.5	13.6	NA	NA
ZSM 116/2021	ACZCV 0030	PT	F	Betampona	25.1	9.8	10.5	3.4	1.5	3.4	2.0	2.6	18.9	8.7	40.5	17.7	11.3	12.5	NA	NA
ZSM 117/2021	ACZCV 0025	PT	M	Betampona	22.4	8.3	9.1	3.1	1.8	2.5	1.9	2.4	19.3	9.0	41.2	17.7	12.1	12.6	5.1	2.4

Table 2. Comparative numerical parameters of analyzed advertisement calls of frogs in the subgenus *Laurentomantis*. Values are provided as range followed by mean ± standard deviation in parentheses.

species	locality	call duration [ms]	note duration [ms]	notes per call	note repetition rate within call [notes/second]	pulses per note	dominant frequency [Hz]
G. horridus	Montagne d'Ambre	543–618 (585.1±27.4)	6–12 (9.4±1.7)	17–19 (18.1±0.8)	29.4–31.0 (29.9±0.7)	2–4 (3.0±0.5)	1172–1369 (1264±71)
G. ranjomavo	Manarikoba Forest	1780–2526 (2237.9±255.6)	10–19 (13.6±3.1)	23–33 (29.0±3.2)	10.8–14.2 (12.7±1.0)	1	2348–3204 (2909±355)
G. striatus	Marojejy	439–1360 (848.5±319.1)	3–7 (5.1±0.8)	17–45 (29.8±8.4)	25.6–33.5 (31.1±3.2)	1	3768–4153 (3983±161)
G. marokoroko	Vohidrazana	557–1203 (968.7±357.7)	10–26 (17.4±5.0)	12–28 (21.0±8.2)	18.2–19.6 (18.8±0.6)	2–5 (3.3±0.9)	2411–2476 (2444±28)
G. malagasius	Vohiparara/ Ranomafana	360–465 (428.4±37.2)	8–17 (12.6±2.9)	8–10 (9.6±0.8)	19.8–21.1 (20.5±0.5)	2–10 (6.2±2.1)	2606–3516 (3127±254)
G. malagasius	Manombo	348–446 (386.3±34.5)	9–16 (12.2±1.9)	16–20 (17.5±1.6)	42.7–45.5 (44.7±1.1)	2–9 (5.5±1.9)	2916–3192 (3041±107)
G. matsilo **sp. nov.** (lineage A)	Betampona	355–660 (552.3±88.0)	13–21 (16.2±2.5)	8–15 (12.6±1.9)	20.8–21.6 (21.2±0.3)	6–10 (6.9±1.1)	3090–3434 (3200±116)
G. fiharimpe **sp. nov.** (lineage B)	Ankeniheny	609–935 (798.8±110.8)	7–9 (7.8±0.8)	11–19 (15.5±2.4)	14.9–25.6 (18.3±3.9)	1	3638–3723 (3676±23)
G. fiharimpe **sp. nov.** (lineage B)	Andasibe	840–1503 (1179.3±232.7)	5–12 (7.6±2.2)	26–43 (36.9±7.3)	18.2–37.4 (25.5±6.5)	1	3649–4078 (3796±157)
G. fiharimpe **sp. nov.** (lineage B)	Vohidrazana	950–1764 (1405.6±290.4)	2–7 (4.3±1.7)	26–52 (40.7±11.5)	18.8–54.1 (32.1±13.1)	1	3726–3913 (3781±68)
G. fiharimpe **sp. nov.** (lineage B)	Ambatolahy/Ranomafana	916–1162 (1073.9±86.0)	4–7 (6.2±0.9)	27–34 (30.6±2.3)	25.6–28.0 (26.8±1.0)	1	3305–3736 (3523±145)
G. oelkrugi **sp. nov.** (lineage C)	Ambodivoangy	279–504 (350.9±88.4)	3–10 (6.8±1.3)	13–25 (16.9±4.4)	43.5–54.1 (46.5±4.0)	1–4 (2.9±0.7)	3266–3882 (3539±190)

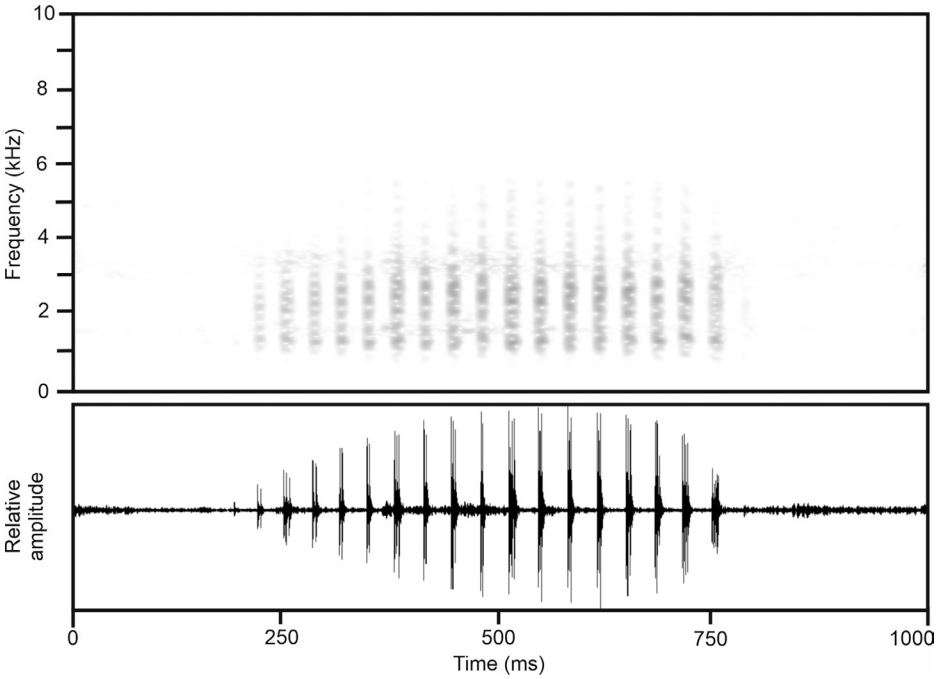

Figure 6. Audiospectrogram and corresponding oscillogram of one advertisement call of *Gephyromantis horridus* (ZSM 126/2018) recorded on 11 December 2017 in Montagne d'Ambre National Park.

genetic lineages (A–D) without scientific names. Of these, lineages A, B and C concordantly differed by a strong mitochondrial divergence (with uncorrected pairwise differences in the 16S gene > 4%), by a lack of haplotype sharing in two nuclear-encoded genes, and by temporal characteristics of advertisement calls. Only lineages B and D have limited haplotype sharing in one nuclear-encoded gene, but all four lineages have several morphological characters that enable their identification. This combined evidence suggests that lineages A, B, C and D represent biologically distinct species, which will be formally named in the respective species accounts in the following.

Gephyromantis horridus (Boettger, 1880)

Hemimantis horrida Boettger, 1880: 282

Note. This species was described based on a juvenile specimen from Nosy Be, and a second specimen (an adult female) from the type locality has previously been reported by Glaw and Vences (2011). Pictures of both specimens are shown in Fig. 4; measurements are given in Vences et al. (2002) and Glaw and Vences (2011), respectively. The Tsaratanana population included in this species by Vences et al. (2002) is assigned to *G. ranjomavo* based on genetic data, and the only locality beside Nosy Be currently accepted for *G. horridus* is therefore Montagne d'Ambre. Measurements of the first male known from the Montagne d'Ambre population are given in Table 1 and photos in life of various individuals from this locality are included in Fig. 5. The male is characterized by very prominent femoral glands of the same color as the surrounding skin of the ventral thigh, composed of approximately six large gland granules as recognizable in external view (Fig. 5). The male has no tibial gland. According to the measurements reported in Vences et al. (2002), Glaw and Vences (2011) and this study, SVL is 33.0–35.4 mm in two females and 33.5 in a male from Montagne d'Ambre, and 33.7 mm in a female from Nosy Be. The advertisement call of this species was previously unknown, and is described in the following for the Montagne d'Ambre population.

Call. The advertisement call of specimen ZSM 126/2018 was recorded at 20:20–21:00 on 11 December 2017 on a muddy bank beside a very slow segment of a stream on the west slope of Montagne d'Ambre (12.5915°S, 049.1372°E, 939 m a.s.l.; air temperature not available). It consists of a series of short, pulsed notes (Fig. 6). They were extremely difficult to localize in the field due to their low amplitude. There is amplidude modulation within each call, with call energy being greatest at approximately 70% of the call's duration, with initial notes being the least energetic ones. Within calls, notes are repeated at very constant intervals. Each note contains few clearly countable pulses repeated at an approximate rate of 500 pulses/second. Within regular call series, calls are emitted in rapid succession. Numerical parameters of 9 analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 543–618 ms (585.1 ± 27.4 ms); note duration 6–12 ms (9.4 ± 1.7 ms); number of notes per call 17–19 (18.1 ± 0.8); note repetition rate within calls 29.4–31.0 notes/second (29.9 ± 0.7 notes/second); pulses per note 2–4 (3.0 ± 0.5); dominant frequency 1172–1369 Hz (1264 ± 71 Hz); prevalent bandwidth 900–3500 Hz. Calls were emitted in regular call series at a rate of approximately 41 calls/minute.

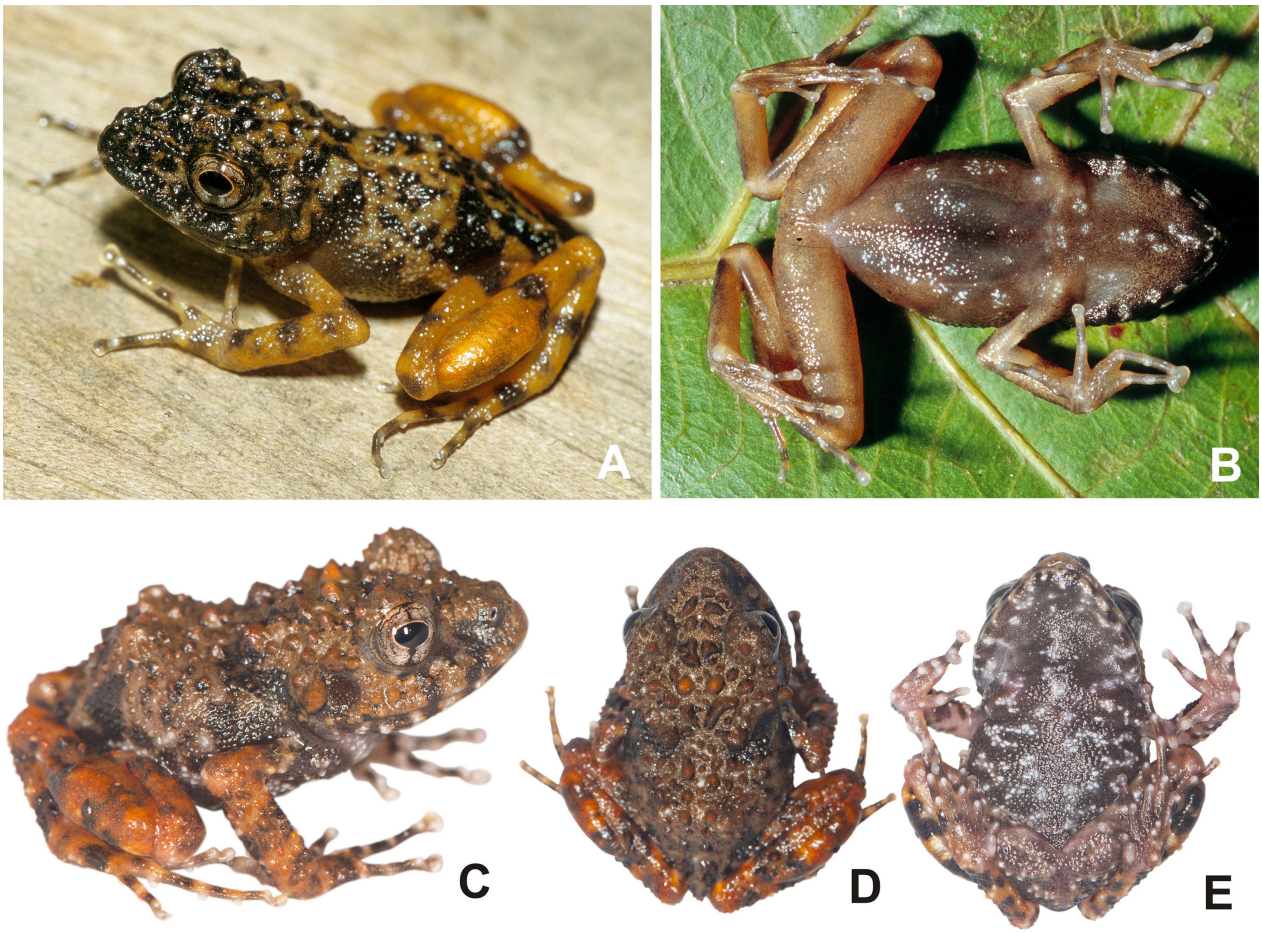

Figure 7. Adult males of *Gephyromantis ranjomavo* in life. **A** Dorsolateral and **B** ventral view of the holotype, ZSM 222/2005 (FGZC 2843) from Marojejy National Park. **C**–**E** Dorsolateral, dorsal and ventral views of specimen ZSM 60/2016 (MSZC 0163) from Ampotsidy, attributed to *G. ranjomavo* but genetically divergent. Not to scale.

Gephyromantis ranjomavo Glaw and Vences, 2011

Gephyromantis ranjomavo Glaw and Vences, 2011: 122

Note. This species was previously known from two specimens (Glaw and Vences 2011), one from the Marojejy Massif and another without precise locality data. As redefined here, the species includes a second deep genetic lineage, expanding the species' range into the southwestern and western foothills of the Tsaratanana Massif (localities Ampotsidy and Manarikoba Forest on the western slope). Morphometric measurements of one newly collected male specimen (ZSM 60/2016 from Ampotsidy) are given in Table 1, and color in life of this individual is shown in Fig. 7. Compared to its sister species *G. horridus* (see data above), both the holotype from Marojejy (Glaw and Vences 2011) and the male from Ampotsidy have relatively small femoral glands without externally visible large gland granules. A tibial gland is present in all specimens. According to the measurements reported in Vences et al. (2002), Glaw and Vences (2011) and this study, male SVL is 23.5 mm in Marojejy, and 25.8–28.1 mm in the remaining specimens; females are unknown.

Call. We redescribe the advertisement call genetically assignable to *Gephyromantis ranjomavo* recorded on 17 February 1997 at Manarikoba Forest, Tsaratanana Strict Nature Reserve (air temperature 17.5°C; Vences et al. 2006: CD2, track 27). The call (previously described by Vences et al. 2002) consists of a long multinote call, usually emitted in series at regular intervals and fast succession (Fig. 8). Slight amplitude modulation is recognizable within calls, with notes at the beginning and the end of the call having lower energy. Notes consist of a single pulse, but in a few notes some diffuse substructure is recognizable. Frequency modulation is absent in notes. Numerical call parameters of 7 newly analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 1780–2526 ms (2237.9 ± 255.6 ms); note duration 10–19 ms (13.6 ± 3.1 ms); number of notes per call 23–33 (29.0 ± 3.2); note repetition rate within calls 10.8–14.2 notes/second (12.7 ± 1.0 notes/second); call repetition rate within call series approximately 14–15 calls/minute; dominant frequency 2348–3204 Hz (2909 ± 355 Hz); prevalent bandwidth 2000–3800 Hz.

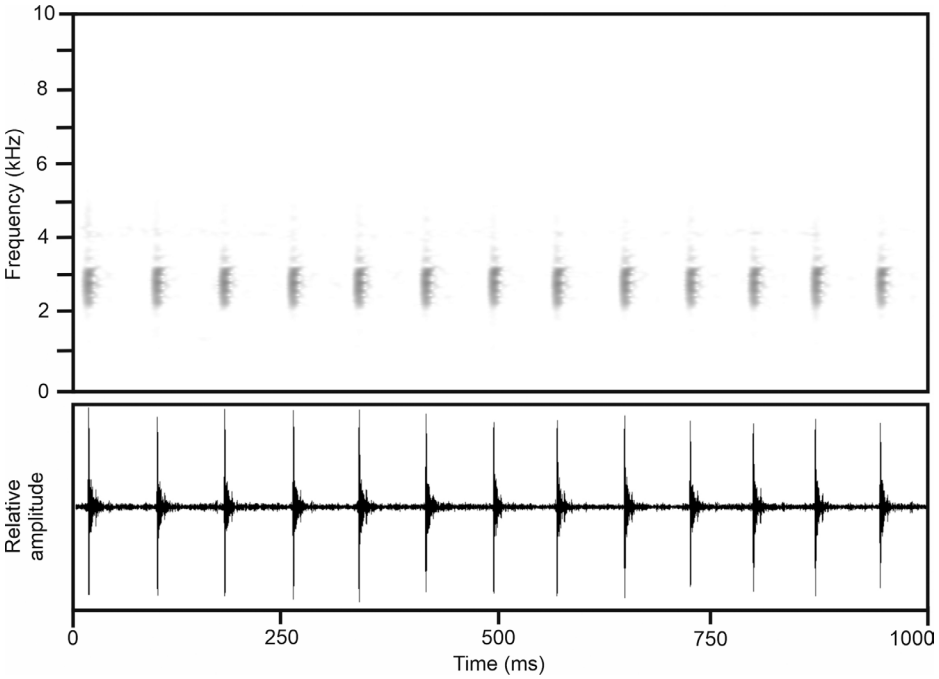

Figure 8. Audiospectrogram and corresponding oscillogram of a 1000 ms section of one advertisement call of *Gephyromantis ranjomavo* recorded on 17 February 1997 at Manarikoba Forest. Recording band-pass filtered at 1000–7500 Hz.

Gephyromantis striatus (Vences, Glaw, Andreone, Jesu and Schimmenti, 2002)

Mantidactylus striatus Vences, Glaw, Andreone, Jesu and Schimmenti, 2002: 203

Note. This species was described from lower elevations of the Marojejy Massif, and specimens from Tsararano and Masoala were assigned to the species based on morphology (Vences et al. 2002). We provide the first molecular data for specimens collected at two sites on the Masoala Peninsula, confirming that these populations are conspecific with those from Marojejy (Fig. 1). *G. striatus* is defined by its rather weakly tubercular dorsum and an incomplete (posterior) light vertebral stripe (Fig. 9). A tibial gland is absent. Based on morphometric data in Vences et al. (2002), SVL ranges from 22.2–23.8 mm in males and 23.9–26.9 mm in females.

Call. The advertisement call was recorded on 22 February 1995 at Marojejy National Park (air temperature 25.0°C; Vences et al. 2006: CD2, track 29). It consists of a moderately long multinote call of variable duration, usually emitted in series at rather regular intervals (Fig. 10). Slight amplitude modulation is recognizable within calls, with notes at the beginning and the end of the call having lower energy. Notes are very short and appear to consist of two pulses, which are largely fused. They are usually repeated at regular intervals within calls, but might be spaced more narrowly at the beginning and end of a call. Frequency modulation is absent. Numerical call parameters of 23 analyzed calls emitted by 2 individuals are as follows (range followed by mean ± standard deviation in parentheses): call duration 439–1360 ms (848.5 ± 319.1 ms); note duration 3–7 ms (5.1 ± 0.8 ms); number of notes per call 17–45 (29.8 ± 8.4); note repetition rate within calls 25.6–33.5 notes/second (31.1 ± 3.2 notes/second); call repetition rate within call series approximately 22–27 calls/minute; dominant frequency 3768–4153 Hz (3983 ± 161 Hz); prevalent bandwidth 2300–5200 Hz.

Gephyromantis marokoroko Hutter, Andriampenomanana, Andrianasolo, Cobb, Razafindraibe, Abraham and Lambert, 2022

Gephyromantis marokoroko Hutter, Andriampenomanana, Andrianasolo, Cobb, Razafindraibe, Abraham and Lambert, 2022: 486

Note. A recently described species from the Northern Central East of Madagascar (Hutter et al. 2022) that is genetically and morphologically distinct by a reddish iris, gray to whitish vocal sacs and large femoral glands with 7–8 distinct, externally visible gland granules, and a dorsal skin texture consisting mainly of sharp ridges rather than of rounded tubercles (Fig. 11). A tibial gland is absent. According to the original description, SVL is 24.0–27.0 mm in males and 23.9–24.6 mm in females. The species is so far only known from several sites near Andasibe: Belakato in Andasibe-Mantadia National Park, Vohimana, Vohidrazana (type locality) and Tavalobe near Vohidrazana. We reanalyzed the call of *G. marokoroko* to ensure full comparability with the call descriptions of all other *Laurentomantis*.

Call. The advertisement call was recorded on 6 January 2016 at Vohidrazana (air temperature 20.4°C; call voucher KU 343230 [CRH 1110]). It consists of a series of short distinctly pulsed notes of variable duration (Fig.

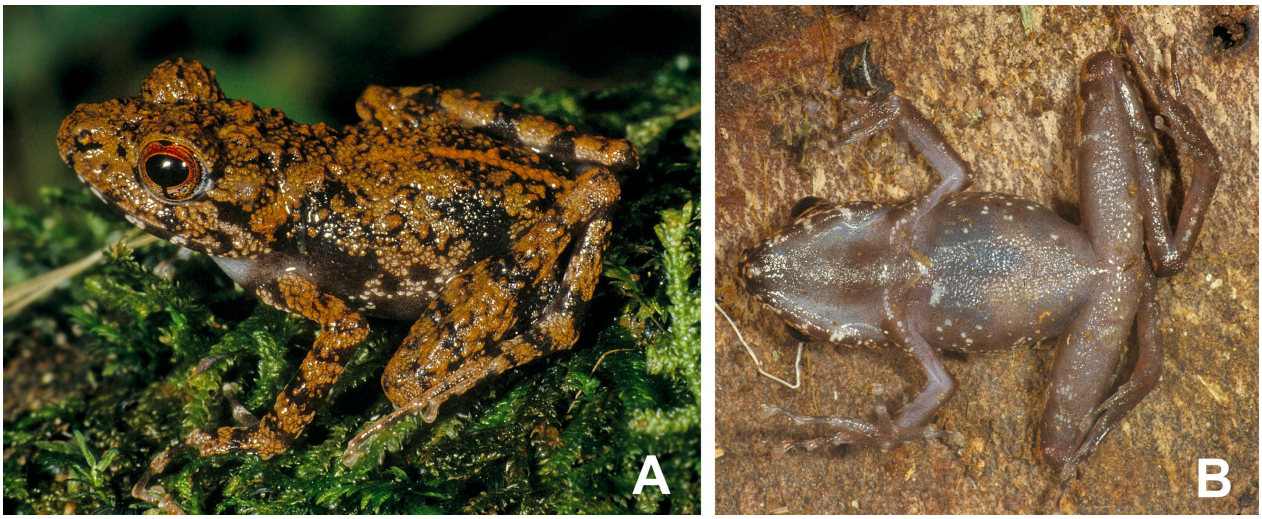

Figure 9. Adult male of *Gephyromantis striatus* from Marojejy National Park in life: **A** dorsolateral and **B** ventral view; photographed in 1994 (individual not reliably attributable to a voucher specimen). Not to scale.

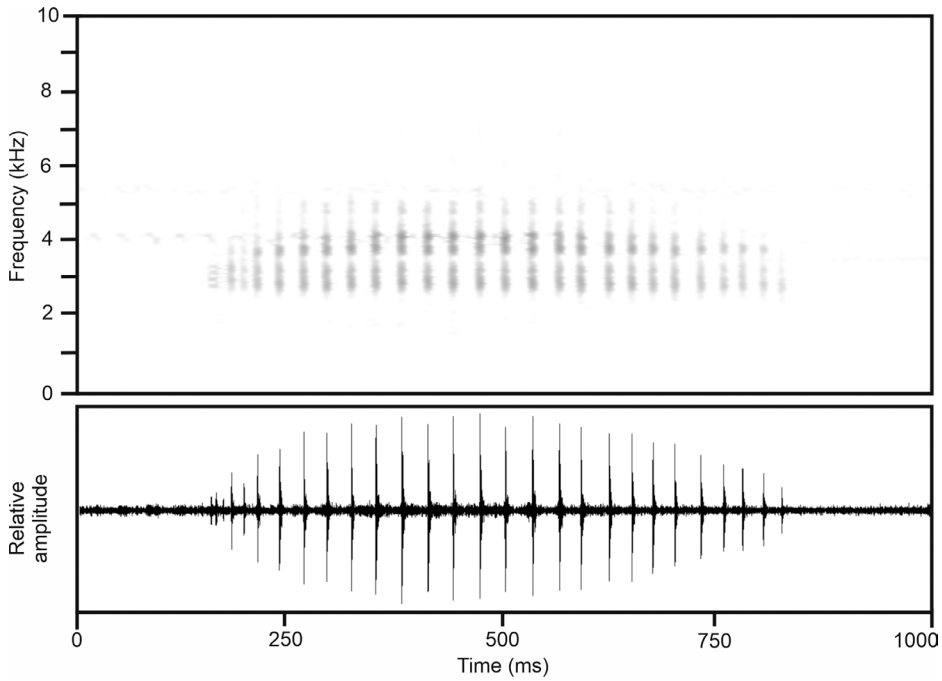

Figure 10. Audiospectrogram and corresponding oscillogram of one advertisement call of *Gephyromantis striatus* recorded on 22 February 1995 at Marojejy National Park. Recording band-pass filtered at 1500–7500 Hz.

12). There is recognizable amplidude modulation within each call, with call energy being greatest at approximately 60% of the call's duration, with initial notes being the least energetic ones. Within calls, notes are repeated at constant intervals. Each note contains a certain number of clearly separated pulses repeated at an approximate rate of 150–170 pulses/second. Numerical parameters of 3 analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 557–1203 ms (968.7 ± 357.7 ms); note duration 10–26 ms (17.4 ± 5.0 ms); number of notes per call 12–28 (21.0 ± 8.2); note repetition rate within calls 18.2–19.6 notes/second (18.8 ± 0.6 notes/second); pulses per note 2–5 (3.3 ± 0.9); dominant frequency 2411–2476 Hz (2444 ± 28 Hz), with a second peak of almost equal energy at around 3600–3700 Hz; prevalent bandwidth 1000–5200 Hz. Call repetition rate in short call series (containing 3 calls) approximately 18–20 calls/minute.

Gephyromantis malagasius (Methuen and Hewitt, 1913)

Microphryne malagasia Methuen and Hewitt, 1913b: 55
Trachymantis malagasia ventrimaculatus Angel, 1935: 205; syn. nov.

Note. As discussed above, we redefine *G. malagasius* based on molecular data from the holotype as containing those frogs previously considered as *G. ventrimaculatus* (e.g., Blommers-Schlösser and Blanc 1991; Vences et

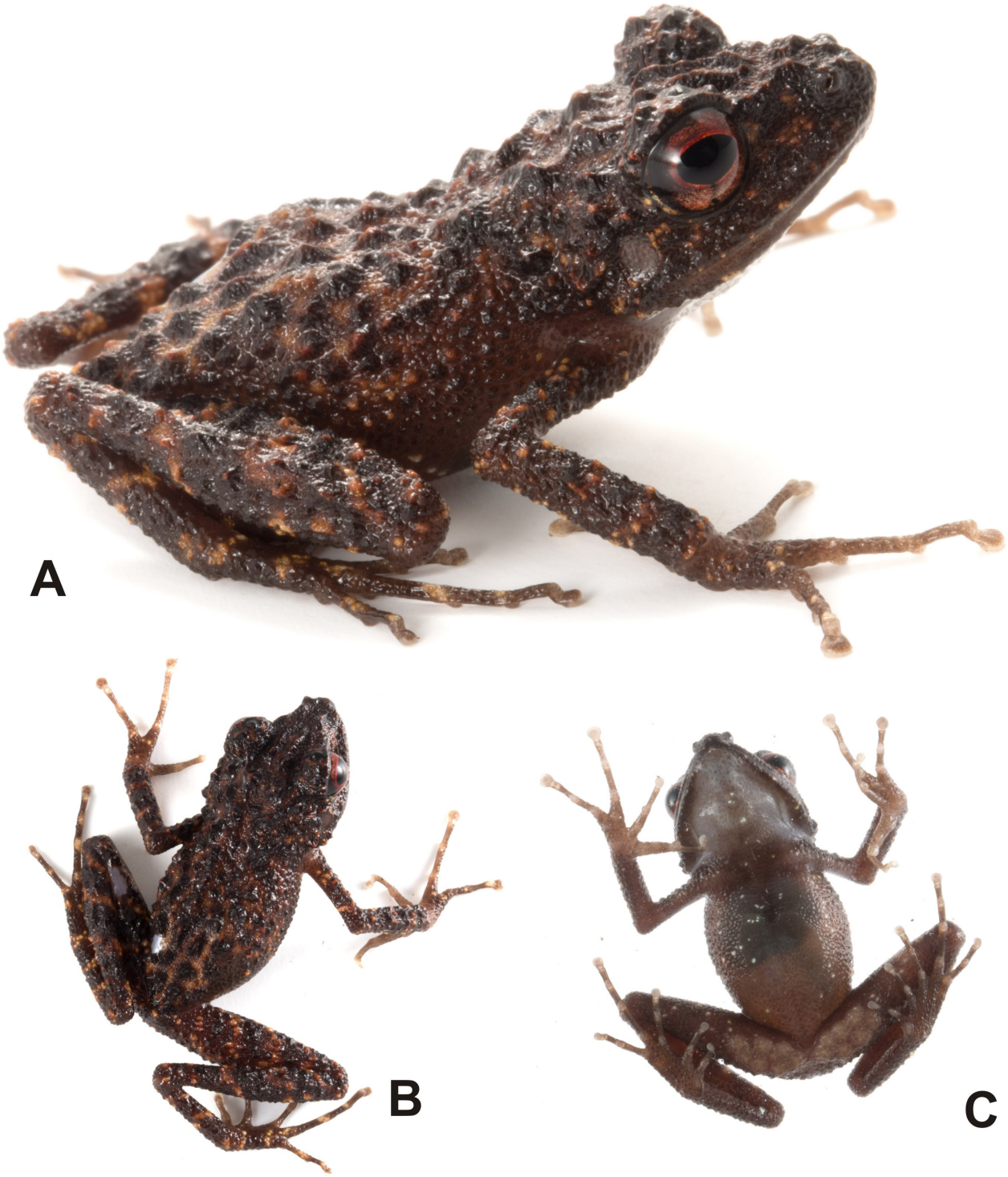

Figure 11. Adult male of *Gephyromantis marokoroko* (KU 343230) from Vohidrazana in life, in **A** dorsolateral, **B** dorsal and **C** ventral view.

al. 2002; Glaw and Vences 2007). These frogs are easily recognizable by their reddish-brown dorsum with highly expressed tubercular skin texture, and a highly contrasted ventral color with grayish to bluish reticulations on a deep black ground color (Fig. 13). A tibial gland is absent. As hypothesized in the Molecular Phylogenetics section, we assume this typical color pattern has faded in the holotype of *Microphryne malagasia* while its general morphology roughly agrees with the morphology of the other specimens assigned to the species, despite being of smaller size (Fig. 14). Vences et al. (2002) discussed the mention in the original description (Methuen and Hewitt 1913b) of "large white blotches" present on the "hidden surface of the thighs and tibiae", and interpreted this pattern as indicative of the reddish areas in life present on those frogs they assigned to *G. malagasius*. However, the described pattern might as well correspond to the contrasted markings found on the ventral side of the specimens previously considered as *G. ventrimaculatus*, which might have persisted in the hidden (not light-ex-

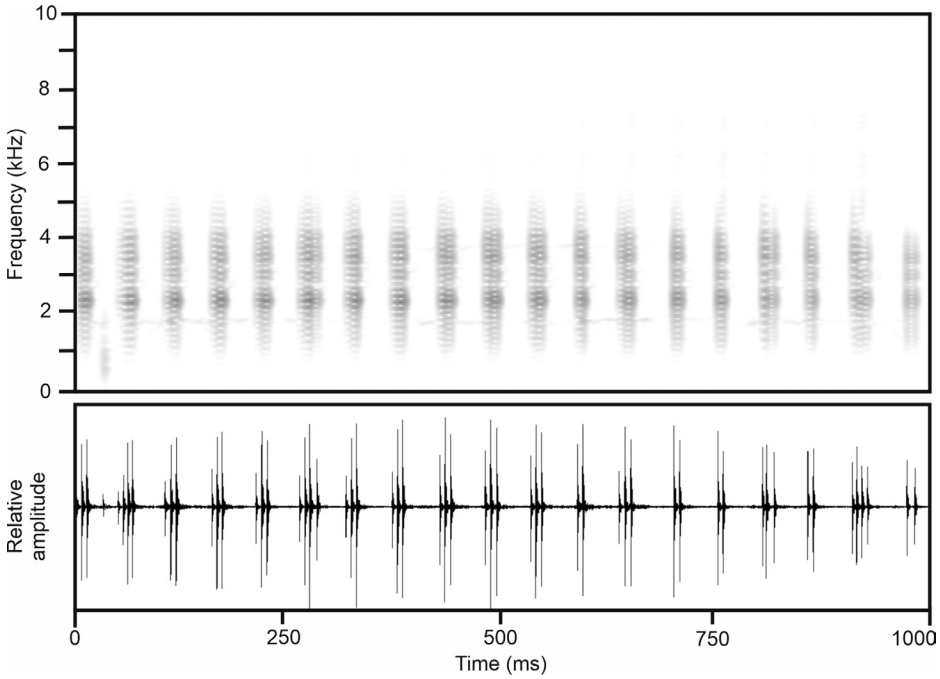

Figure 12. Audiospectrogram and corresponding oscillogram of a 1000 ms section of an advertisement call of *Gephyromantis marokoroko* recorded on 6 January 2016 at Vohidrazana.

posed) parts of the limbs at the time of the original description.

Vences et al. (2002) also provided information on the femoral gland of the *G. malagasius* holotype, which according to them consists of 1–2 granules (examined in external view), while the femoral glands of specimens assigned to *G. ventrimaculatus* were found to consist of nine granules. For this study, we unfortunately were not able to examine the gland of the *malagasius* holotype in internal view, but a detailed look in external view (Fig. 13) reveals a rounded, well-defined structure, which may consist of several granules (more than the 1–2 previously reported), and clearly differs from the corresponding gland structures typical for specimens of lineage B where one or two single granules are arranged longitudinally on the ventral thigh; however, at least lineages C and D also have glands composed of more (4–7) granules, suggesting that femoral gland characteristics cannot be used to unambiguously allocate the *malagasius* holotype to a genetic lineage. Summarizing morphometric measurements of Vences et al. (2002) and the present study, males measure 23.0–27.0 mm, females measure 29.1–29.8 mm, and the *malagasius* holotype measures 20.2 mm SVL. We here provide bioacoustic data from Ranomafana National Park (Vohiparara) and from a second locality, Manombo.

Call. The advertisement call of *G. malagasius* recorded at Vohiparara (Vences et al. 2006, CD2, track 30) from ZFMK 62281 consists of a series of very short pulsed notes and is emitted in series at regular intervals (Fig. 15). There is amplidude modulation within each call, with call energy increasing from the beginning of the call reaching the maximum amplitude at about 40% of its duration and from there decreasing towards its end. Pulses within notes are partly fused, but clearly countable. Within calls, notes are repeated at regular intervals. Numerical parameters of 7 analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 360–465 ms (428.4 ± 37.2 ms); note duration 8–17 ms (12.6 ± 2.9 ms); number of notes per call 8–10 (9.6 ± 0.8); note repetition rate within calls 19.8–21.1 notes/second (20.5 ± 0.5 notes/second); pulses per note 2–10 (6.2 ± 2.1); call repetition rate within call series approximately 17–18 calls/minute; dominant frequency 2606–3516 Hz (3127 ± 254 Hz); prevalent bandwidth 1800–6000 Hz.

A second recording from 23 February 2007 (Manombo; air temperature estimated at 23–25°C) consists of a series of pulsed notes and is emitted in series at regular intervals (Fig. 16). There is amplidude modulation within each call, with call energy increasing from the beginning of the call reaching the maximum amplitude at about 60% of its duration and from there decreasing towards its end. Pulses within notes are partly fused, but in most notes distinct pulses are recognizable and countable. Within calls, notes are repeated at regular intervals at a high rate. Numerical parameters of 14 analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 348–446 ms (386.3 ± 34.5 ms); note duration 9–16 ms (12.2 ± 1.9 ms); number of notes per call 16–20 (17.5 ± 1.6); note repetition rate within calls 42.7–45.5 notes/second (44.7 ± 1.1 notes/second); pulses per note 2–9 (5.5 ± 1.9); call repetition rate within call series approximately 39–44 calls/minute; dominant frequency 2916–3192 Hz (3041 ± 107 Hz); prevalent bandwidth 1700–5500 Hz.

Distribution. *G. malagasius* as redefined here is known based on genetically confirmed records from (1) the type locality Folohy, (2) Ranomafana, (3) Manombo, and (4) Befotaka-Midongy. Furthermore, morphologically

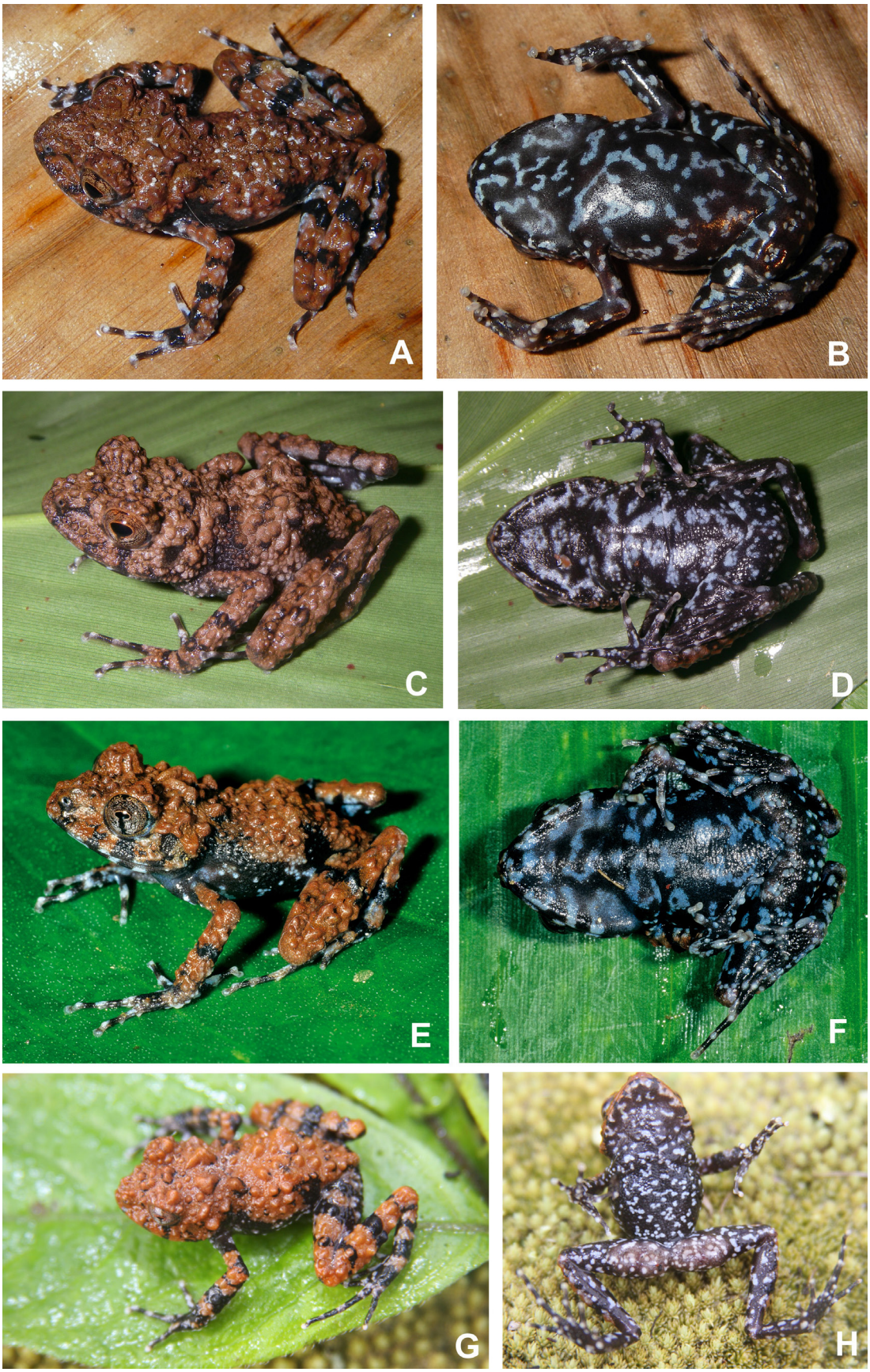

Figure 13. Specimens of *Gephyromantis malagasius* (previously considered as *G. ventrimaculatus*, herein considered to be a junior synonym of *G. malagasius*), in life. **A**, **B** Adult male from Ranomafana in dorsolateral and ventral view, photographed 2006, probably corresponding to voucher specimen ZSM 537/2006 (ZCMV 3362). **C**, **D** Adult male from Manombo, ZSM 2464/2007 (ZCMV 5497) in dorsolateral and ventral view, photographed 2007. **E**, **F** Adult male from Ranomafana (Vohiparara), photographed 1996, possibly corresponding to voucher specimen ZFMK 62281. **G**, **H** Adult male from Ambohitantely, not clearly assignable to a voucher specimen, photographed in 2017 in Ambohitantely Special Reserve. Not to scale.

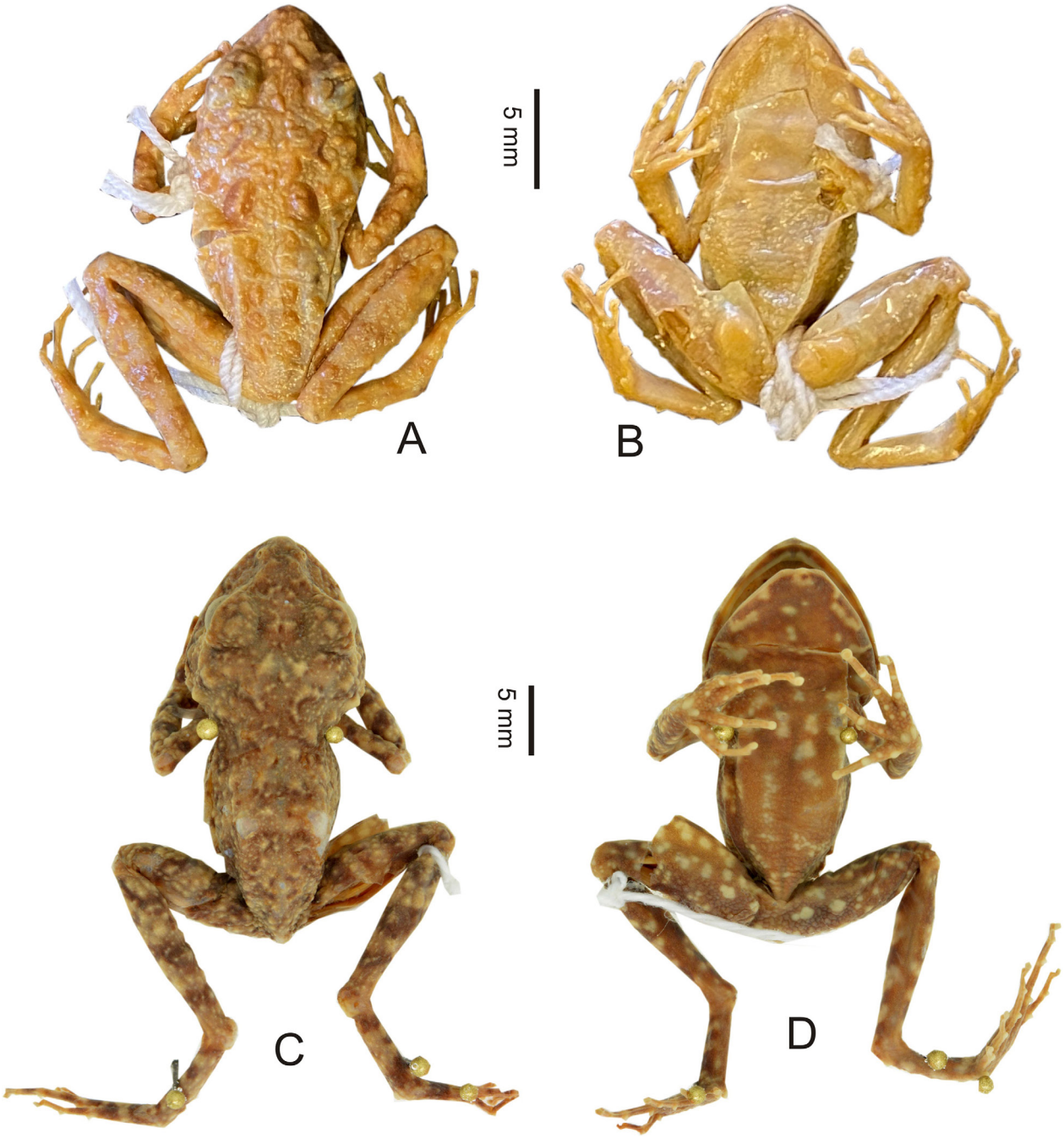

Figure 14. A, **B** Preserved holotype of *Gephyromantis malagasius* (originally named *Microphryne malagasia*; specimen TMP 10076) in dorsal and ventral view. **C**, **D** Preserved lectotype of *Trachymantis malagasia ventrimaculatus* (MNHN 1935.172), herein considered as a junior synonym of *G. malagasius*. Photos C and D by Antoine Fraysse, project MNHN-RECOLNAT (ANR-11-INBS-0004), photographed in 2015 (http://coldb.mnhn.fr/catalognumber/mnhn/ra/1935.173)

identified specimens with the typical ventral pattern are known from (5) Andasibe, (6) Ambohitantely (based on phenotypically identified specimens collected by one of us [APR]; see Fig. 13G,H), and (7) the type locality of the junior synonym *ventrimaculatus* ("Isaka Ivondro, alt. 700 m"), which is located within or very close to the current Andohahela National Park. It is important to mention that the exact location of the type locality Folohy is uncertain. At this site, collections were made by "M. Herschell-Chauvin" in 1911 (Methuen and Hewitt 1913b). Methuen and Hewitt (1913a) name the collector "Monsieur Herschell-Chauvin", probably referring to the English naturalist and photographer Charles Herschell-Chauvin who worked in Tamatave (=Toamasina) in the first years of the 20th century. Methuen and Hewitt (1913a) place the locality Folohy "in the neighbourhood of Tamatave", and also Blommers-Schlösser and Blanc (1991) plot Folohy as near-coastal locality close to Toamasina in their distribution maps. Barbour and Loveridge (1929) locate Folohy "east of Lake Alaotra" for a frog specimen exchanged from the Transvaal Museum. The catalogue of the Museum of Comparative Zoology, Harvard, includes a lemur specimen (MCZ 18740) collected by Frederick Roelker Wulsin in 1915, with the verbatim locality in-

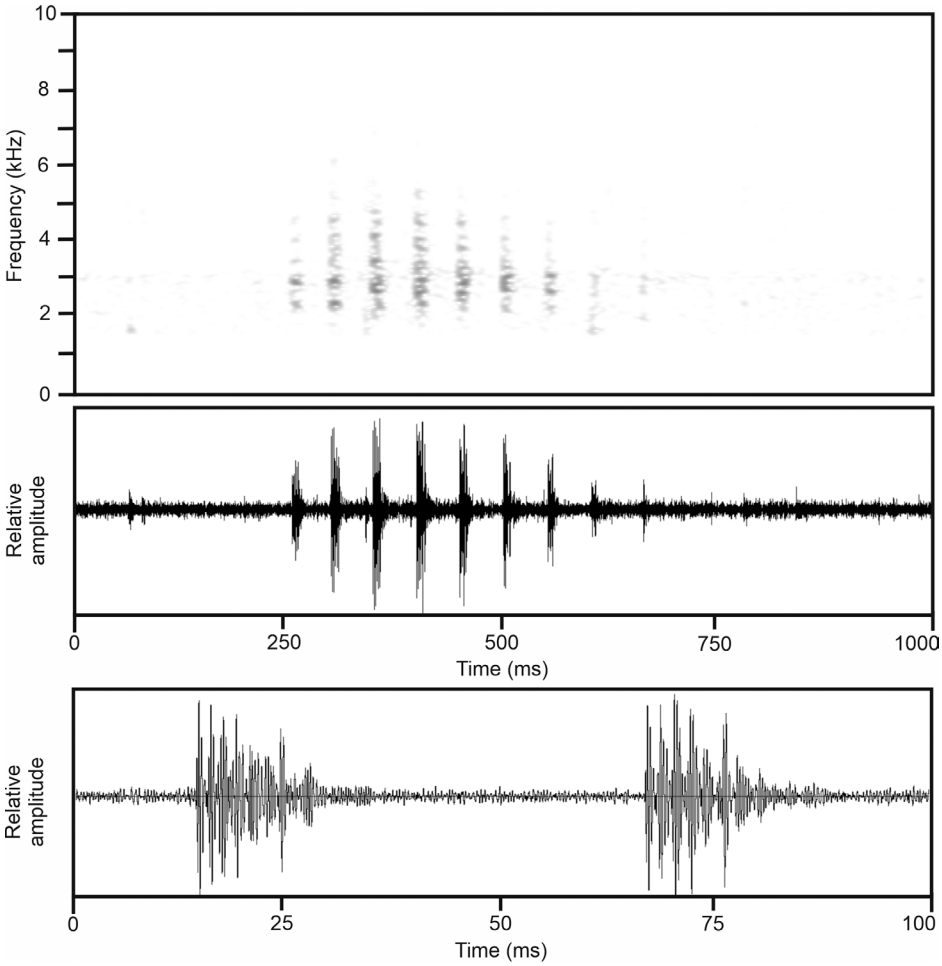

Figure 15. Audiospectrogram and corresponding oscillogram of one advertisement call of *Gephyromantis malagasius* recorded at Vohiparara. The oscillogram below shows a 100 ms section of the call figured above, showing two notes and their repective pulse structure. Recording band-pass filtered at 1500–7500 Hz.

formation "Folohy forest, 100 miles west of Tamatave" (https://www.idigbio.org/portal accessed 19 November 2021), which however is unlikely to be correct as it would place the site onto Madagascar's high plateau, outside the main rainforest area. Along with Blommers-Schlösser and Blanc (1991) we here assume that Folohy refers to a low- or mid-elevation site close to Toamasina.

The occurrence of individuals morphologically corresponding to *G. malagasius* as redefined herein in Andasibe is supported by three records: one voucher specimen collected by Denis Vallan and reported in Vences et al. (2002); one specimen photographed by Daniel S. Moen; and one specimen photographed by Devin Edmonds in the Mitsinjo forest on 4 December 2014 (https://www.inaturalist.org/observations/2315204).

Gephyromantis matsilo sp. nov. (lineage A)

http://zoobank.org/55361232-0291-4652-80F3-B030F0A9-1FBB

Holotype. ZSM 711/2009 (field number ZCMV 7234), adult male, from campsite "Ambatoroma, S II " (a campsite in the Manompana – Befanjana Forest area, approximately at coordinates 16.66°S, 49.59°E; precise elevation unknown), Analanjirofo Region, eastern Madagascar, collected on 19 May 2009 by J.E. Randrianirina.

Paratypes. ZSM 712/2009 (ZCMV 7300), adult female, from campsite "Babitanety, S III" (Manompana – Befanjana Forest), Analanjirofo Region, eastern Madagascar, collected on 20 May 2009 by J.E. Randrianirina; MRSN A5678 (FAZC 13344), adult male, from Sahavontsira (Miorimivalana, Fenerive Est), Analanjirofo Region, eastern Madagascar, collected on 23 January 2006 by F. Andreone, F. Mattioli and J.E. Randrianirina; ZMA 20247 (ZCMV 90), adult male, from Ambohitsara (21.3572°S, 047.8157°E, 294 m a.s.l.), Vatovavy-Fitovinany Region, eastern Madagascar, collected on 21 January 2004 by D.R. Vieites and I. de la Riva.

Etymology. The species epithet is derived from the Malagasy adjective *matsilo* (spiny) and refers to the spiny tubercles on the dorsum of this frog. The name is used as a noun in apposition.

Diagnosis. A member of the subfamily Mantellinae based on the presence of intercalary elements between terminal and subterminal phalanges of fingers and toes (verified

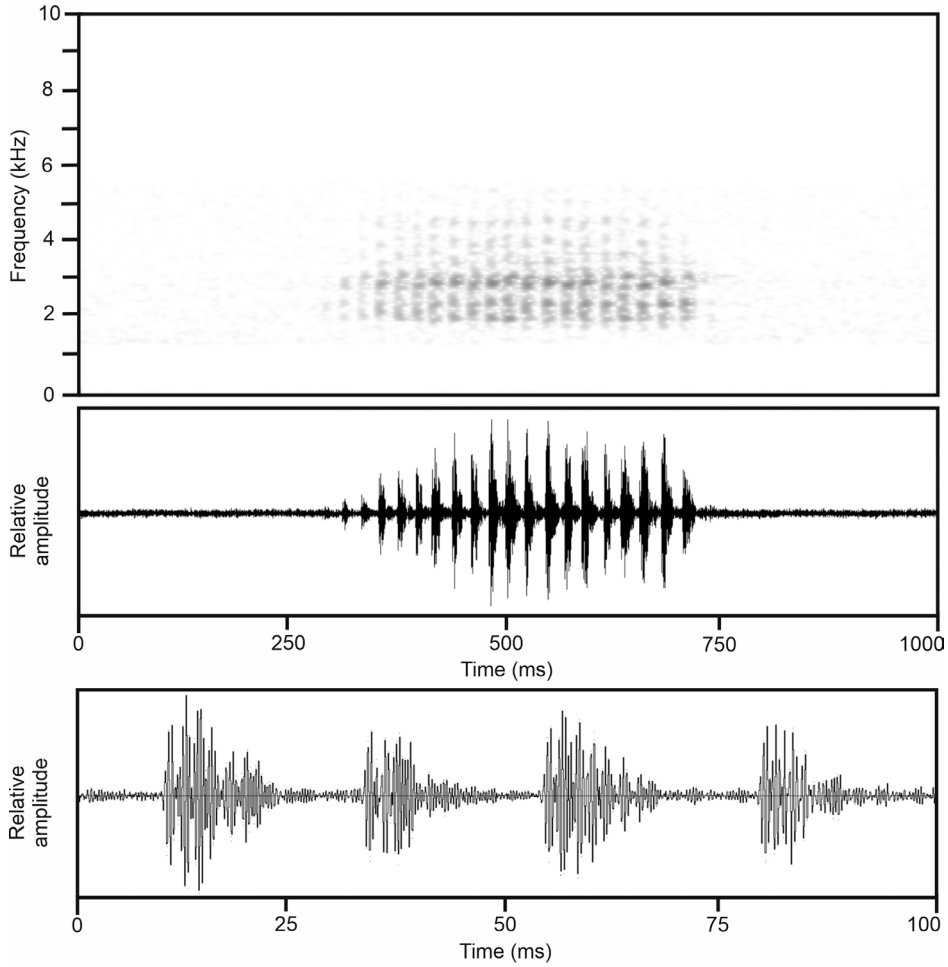

Figure 16. Audiospectrogram and corresponding oscillogram of one advertisement call of *Gephyromantis malagasius* recorded at Manombo. The oscillogram below shows a 100 ms section of the call figured above, showing four notes and their repective pulse structure. Recording band-pass filtered at 1500–6200 Hz.

externally), and on the absence of nuptial pads and presence of femoral glands in males. Assigned to the subgenus *Laurentomantis* in the genus *Gephyromantis* based on the strongly tubercular dorsal skin, absence of foot webbing, completely connected lateral metatarsalia, and molecular phylogenetic relationships. The new species differs from all nominal species of the subgenus *Laurentomantis* as follows: From *G. horridus* by smaller body size (male SVL 21.7–21.9 mm vs. 33.5 mm) and absence of a dorsal pattern of two blackish transverse patches (vs. presence); from *G. ranjomavo* by slightly smaller body size (male SVL 21.7–21.9 mm vs. 23.5–28.1 mm), absence of light brown to orange-brown color covering limbs dorsally (vs. presence), and absence of a tibial gland (vs. presence); from *G. striatus* by absence of a vertebral stripe posteriorly on dorsum (vs. presence) and a more strongly tubercular dorsal skin; from *G. malagasius* (as redefined herein) by absence of a distinct bluish gray pattern on a dark venter (vs. presence); and from *G. marokoroko* by a more coarsely tubercular dorsal skin, presence of red color ventrally on limbs (vs. absence), absence of orange spots and vermiculations on dorsum (vs. presence), and absence of gray to whitish color on vocal sac (vs. presence). Also distinguished from *G. horridus* and *G. striatus* by longer notes in advertisement calls (13–21 vs. 3–12 ms). Furthermore, from all nominal *Laurentomantis* species distinguished by the presence of bright red color in the inguinal region and probably ventrally on limbs and posterior belly in life (see. Fig. 17 where this color is recognizable in the inguinal region) (vs. absence), and by a substantial genetic divergence (>8% uncorrected pairwise distance in the 16S gene). For a distinction from the other new species described in the following (lineages B, C and D), see the diagnoses in the respective species accounts below.

Description of the holotype. Adult male in good state of preservation (Fig. 18). SVL 21.9 mm, for other measurements see Table 1. Body slender; head slightly longer than wide, wider than body; snout rounded in dorsal and lateral views; nostrils directed laterally, distinctly protuberant, much nearer to tip of snout than to eye; canthus rostralis concave; loreal region distinctly concave; tympanum distinct, rounded, 77% of eye diameter; no supratympanic fold except some elevated skin folds directly encircling the tympanum dorsally; tongue ovoid, distinctly forked posteriorly; vomerine teeth absent; choanae rounded; maxillary teeth present. Dermal fold along the posterior part of the lower jaws (the inflatable parts of the vocal sac) weakly expressed. Arms slender,

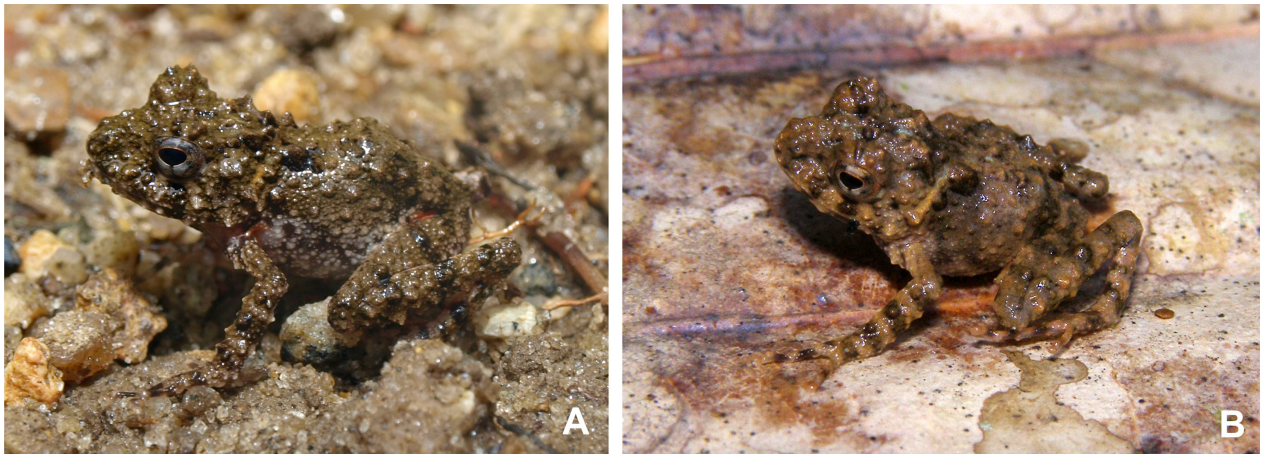

Figure 17. Specimens of *Gephyromantis matsilo* **sp. nov.** (lineage A) in life in dorsolateral view. **A** Specimen from Ambodiriana probably corresponding to tissue sample PSG 1015. **B** Specimen from Antanambe, probably corresponding to tissue sample PSG 49.Vouchers not collected (photo by P.S. Gehring).

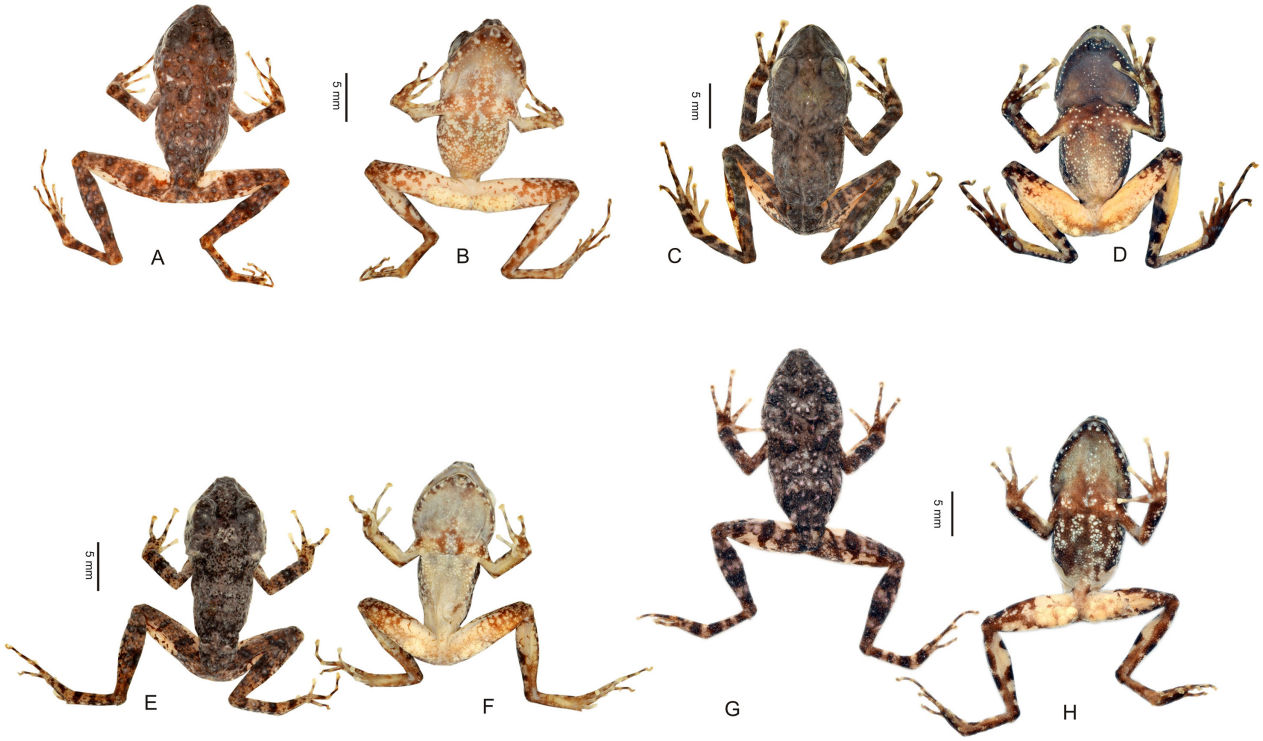

Figure 18. Preserved holotype specimens of the four new *Gephyromantis* species in dorsal and ventral view. **A**, **B** *G. matsilo* **sp. nov.** (lineage A), ZSM 711/2009 (ZCMV 7234) from Ambatoroma, Befanjana Forest. **C**, **D** *G. fiharimpe* **sp. nov.** (lineage B), ZSM 164/2016 (FGZC 5181) from Mandraka. **E**, **F** *G. oelkrugi* **sp. nov.**, ZSM 314/2010 (FGZC 4220) from Ambodivoangy. **G**, **H** *G. portonae* **sp. nov.** (lineage D), ZSM 115/2021 (ACZCV 0032) from Betampona.

subarticular tubercles single; outer and inner metacarpal tubercles weakly expressed, not prominent; fingers without webbing; relative length of fingers 1 < 2 = ≤ 4 < 3, second finger distinctly shorter than fourth finger on right hand, almost of same length on left hand; finger discs enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaching nostrils when hindlimb is adpressed along body; lateral metatarsalia connected; inner metatarsal tubercle distinct, outer metatarsal tubercle small but recognizable; webbing between fingers and toes absent; relative toe length 1 < 2 < 5 = 3 < 4. Third toe of same length as fifth toe. Toe discs enlarged. Skin on upper surface granular, with rather indistinct ridges especially on anterior dorsum, and many smaller irregularly distributed tubercles on head, eyes, and dorsum. Ventral skin smooth on throat, chest and limbs, slightly granular on posterior belly. Femoral glands well delimited and distinctly recognizable from external view. No tibial glands.

After twelve years in preservative, dorsal coloration of head and body uniformly dark brown, with darker crossbands on hind- and forelimbs. Posterodorsal surface of

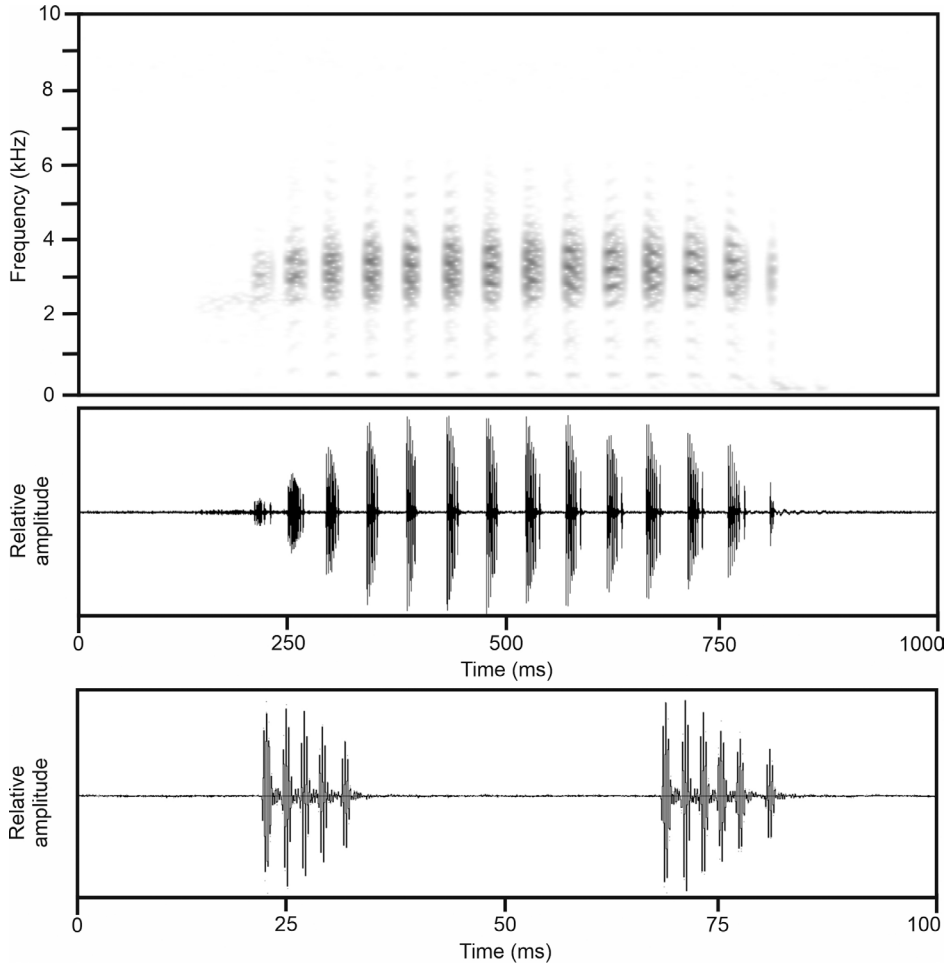

Figure 19. Audiospectrogram and corresponding oscillogram of one advertisement call of *Gephyromantis matsilo* **sp. nov.** recorded on 12 December 2007 at Betampona. The oscillogram below shows a 100 ms section of the call figured above, showing two notes and their respective pulse structure.

thigh with a small pigmentless patch near the knee joint, this area presumably corresponding to reddish color in life. Ventrally cream, with a rather faint and irregular brown marbling, which is more contrasted on the ventral surface of the hindlimbs.

Variation. All photographed and examined specimens of *G. matsilo* lack a tibial gland. The male ZMA 20247 from Ambohitsara agrees with the morphology of the specimens examined from the northern localities in the Befanjana forest, including body size, and a more spiny-granular dorsal skin compared to specimens of lineage B. The female ZSM 712/2009 is distinctly larger than the two measured males (SVL 24.9 mm vs. 21.7–21.9 mm).

Call. The advertisement call (Fig. 19) was recorded on 12 December 2007 at Vohitsivalana, RNI Betampona (air temperature 20°C; Rosa et al. 2011: track 24; sequence HM364637 from specimen FAZC 13977). It consists of a series of short distinctly pulsed notes. There is considerable amplidude modulation within each call, with call energy being greatest at approximately the middle of the call, with initial notes being the least energetic. Within calls, notes are repeated at very constant intervals. Each note contains several clearly separated pulses repeated at an approximate rate of 500 pulses/second. In some notes, the terminal pulse is separated by a slightly larger interval from preceding pulses. Numerical parameters of 14 analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 355–660 ms (552.3 ± 88.0 ms); note duration 13–21 ms (16.2 ± 2.5 ms); number of notes per call 8–15 (12.6 ± 1.9); note repetition rate within calls 20.8–21.6 notes/second (21.2 ± 0.3 notes/second); pulses per note 6–10 (6.9 ± 1.1); dominant frequency 3090–3434 Hz (3200 ± 116 Hz); prevalent bandwidth 2000–4700 Hz. Calls were emitted more or less isolated or in short call series (containing up to 12 calls) at a rate of 24–31 calls/minute within series.

Distribution and natural history. Based on genetically verified records, the distribution area spans a south-north direction from: (1) Ambohitsara to (2) Betampona, (3) Ambodiriana, (4) two sites in Befanjana, and (5) Sahavontsira. The known elevational range of the species spans from near sea level (Ambodiriana, 53 m a.s.l.) to approximately 294 m a.s.l. (Ambohitsara). Very little is known on the natural history of this species. Despite intensive sampling, only three individuals of this species

have been collected at Betampona, where the most commonly found *Laurentomantis* species is the lineage D. All individuals were collected in rainforest habitat.

Gephyromantis fiharimpe sp. nov. (lineage B)

http://zoobank.org/E8DB6DB0-A807-4F39-9589-81FBFC23E33E

Holotype. ZSM 164/2016 (field number FGZC 5181), adult male, from Mandraka (18.9122°S, 047.9144°E, 1235 m a.s.l.), Analamanga Region, Northern Central East of Madagascar, collected on 5 January 2016 by F. Glaw, D. Prötzel, and L. Randriamanana.

Paratypes. MRSN A6436 (field number PBZT/RJS 1983), adult male, from Vevembe (Site A: camp forêt), Atsimo Atsinanana Region, Southern Central East of Madagascar, collected on 26 October 2007 by J.E. Randrianirina and J. Randriantsoa; UADBA 20646 (FGMV 2002.530), unsexed, ZMA 19421 (FGMV 2002.415), probable female, and ZSM 746/2003 (FGMV 2002.531), adult male, from Ranomafana National Park, Vatovavy-Fitovinany Region, south central eastern Madagascar, all collected 22–24 January 2003 by F. Glaw, M. Puente, L. Raharivololoniaina, M. Teschke (née Thomas), D.R. Vieites; ZFMK 57434, ZFMK 59876, two adult males, from Andasibe, Alaotra-Mangoro Region, Eastern Madagascar, collected between 1–4 January 1994 by F. Glaw and M. Vences; ZFMK 60039, adult male, from Andasibe, Alaotra-Mangoro Region, Eastern Madagascar, collected on 1 February 1995 by F. Glaw. NMBE 233/96, adult male, from Ambohitantely, Analamanga Region, central Madagascar, collected by D. Vallan. The ZFMK and NMBE specimens are included as paratypes despite the lack of associated DNA sequences because they bear a tibial gland and originate from sites where the presence of lineage B was ascertained by genetic data (Fig. 1). KU 340759 (CRH 511), UADBA-CRH 486, KU 340863 (CRH 746), collected at Ranomafana National Park by C. R. Hutter and S. Lambert; KU 340736 (CRH 470) collected at Vohidrazana, Alaotra-Mangoro Region, Eastern Madagascar, by C. R. Hutter and S. Lambert; UADBA uncatalogued (APR 7651) collected at Ambohitantely Special Reserve (Jardin botanique), 1560 m a.s.l., Analamanga Region, central Madagascar, by A.P. Raselimanana in 2007. UADBA uncatalogued (APR 8659), male, collected at Ambatovy-Analamay Forest (18.7989°S, 048.3242°E, 1100 m a.s.l.), Alaotra-Mangoro Region, eastern Madagascar, by A.P. Raselimanana in 2009. UADBA uncatalogued (APR 8448), collected at Maromizaha Forest (18.9757°S, 048.4583°E, 1000 m a.s.l.), Alaotra-Mangoro Region, eastern Madagascar, by A.P. Raselimanana in 2008. UADBA uncatalogued (APR 12214) collected at NAP Anjozorobe-Sahabe (18.4208°S, 047.9438°E, 1305 m a.s.l.), Analamanga Region, central Madagascar, by A.P. Raselimanana in 2016.

Referred specimen. ZFMK 57435, female, from Ankeniheny, Alaotra-Mangoro Region, Eastern Madagascar, collected on 19 February 1994 by F. Glaw, N. Rabibisoa and O. Ramilison. This female specimen with tibial gland (Vences et al. 2002) is not included in the paratype series because no genetic data are available, neither for the specimen nor for the general locality Ankeniheny.

Etymology. The species epithet is derived from the Malagasy words *fihary* (gland) and *fe* (leg) which written together become *fiharim-pe* according to Malagasy grammar. The name makes reference to the tibial gland of the species, and is used as a noun in apposition.

Diagnosis. A member of the subfamily Mantellinae based on the presence of intercalary elements between terminal and subterminal phalanges of fingers and toes (verified externally), and on the absence of nuptial pads and presence of femoral glands in males. Assigned to the subgenus *Laurentomantis* in the genus *Gephyromantis* based on the strongly tubercular dorsal skin, absence of foot webbing, completely connected lateral metatarsalia, and molecular phylogenetic relationships. The new species differs from all nominal species of the subgenus *Laurentomantis* as follows: From *G. horridus* by smaller body size (male SVL 22.0–24.0 mm vs. 33.5 mm), less expressed tubercles and ridges on the dorsum, and absence of a dorsal pattern of two blackish transverse patches (vs. presence); from *G. ranjomavo* by the absence of light brown to orange-brown color covering limbs dorsally (vs. presence), less expressed tubercles and ridges on the dorsum, and possibly slightly smaller body size (male SVL 22.0–24.0 mm vs. 23.5–28.1 mm); from *G. striatus* by absence of a vertebral stripe posteriorly on dorsum (vs. presence) and a more strongly tubercular dorsal skin; from *G. malagasius* (as redefined herein) by absence of a distinct bluish gray pattern on a dark venter (vs. presence), and less expressed tubercles and ridges on the dorsum; from *G. marokoroko* by a somewhat less tubercular dorsal skin, presence of red color ventrally on limbs (vs. absence), absence of orange spots and vermiculations on dorsum (vs. presence), and absence of gray to whitish color on vocal sac (vs. presence). Furthermore, differing from all the aforementioned species by the presence of light reddish color in the inguinal region and ventrally on limbs and posterior belly in life (vs. absence), and from all species except for *G. ranjomavo* by the presence of a tibial gland (vs. absence), and by a substantial genetic divergence (>6% uncorrected pairwise distance in the 16S gene).

According to the molecular phylogeny, *G. fiharimpe* is closely related to *G. matsilo* described above, and may be its sister species. It differs from *G. matsilo* by presence of a tibial gland (vs. absence), shorter note duration in advertisement calls (2–12 ms vs. 13–21 ms), a less tubercular dorsal skin, and an uncorrected genetic distance in the 16S gene of 4.0–5.7%.

For a distinction from the other new species described in the following (lineages C and D), see the diagnoses in the respective species accounts below.

Description of the holotype. Adult male in good state of preservation (Fig. 18), tongue removed as tissue sam-

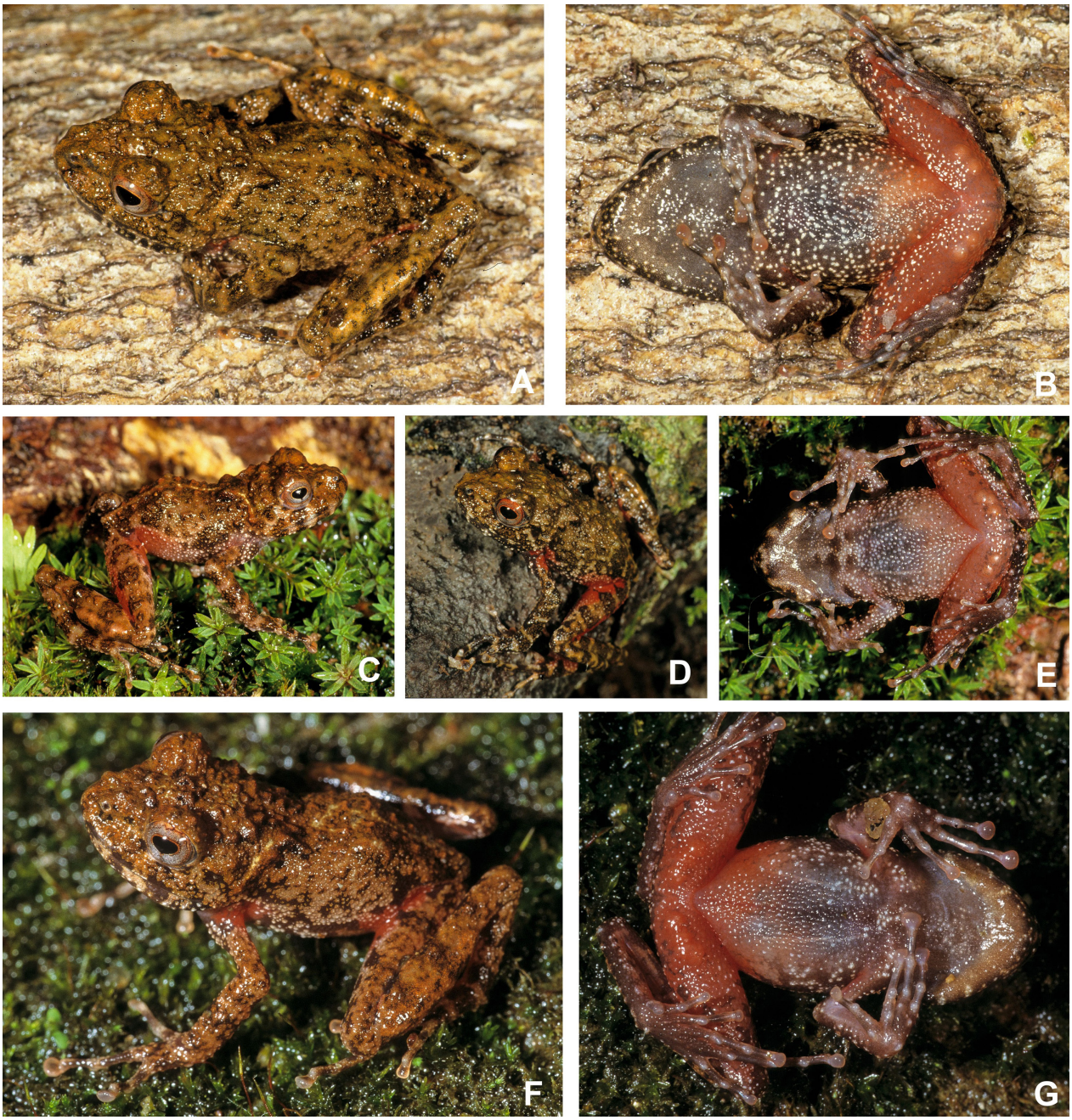

Figure 20. Specimens of *Gephyromantis fiharimpe* **sp. nov.** (lineage B) in life, in dorsolateral and ventral views. **A**, **B** Adult male, probably from An'Ala, photographed 1996. **C**–**E** Adult male from Andasibe, photographed 1994. **F**, **G** Adult male from Ranomafana, photographed 2003. Individuals not reliably attributable to a voucher specimen. Not to scale.

ple for molecular analysis. SVL 23.4 mm, for other measurements see Table 1. Body slender; head longer than wide, as wide as body; snout rounded in dorsal view, subacuminate in lateral view; nostrils directed laterally, slightly protuberant, much nearer to tip of snout than to eye; canthus rostralis rather indistinct, concave; loreal region slightly concave; tympanum distinct, rounded, 53% of eye diameter; supratympanic fold not recognizable, in its place two large tubercles; tongue removed and thus not available for examination; vomerine teeth weakly recognizable, but present in two minuscule aggregations posteromedially to choanae; choanae rounded; maxillary teeth present. Dermal fold along the lower jaws (the inflatable parts of the vocal sac) weakly expressed. Arms slender, subarticular tubercles single; poorly developed outer and inner metacarpal tubercles recognizable; fingers without webbing; relative length of fingers 1 < 2 < 4 < 3, second finger distinctly shorter than fourth finger; finger discs distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaching snout tip when hindlimb is adpressed along body; lateral metatarsalia connected; inner metatarsal tubercle distinct, outer metatarsal tubercle small but recognizable; webbing between fingers and toes absent; relative toe length 1 < 2 < 5 < 3 < 4. Third toe only slightly longer than fifth toe. Toe discs enlarged. Skin on upper surface granular, with rather indistinct ridges and many smaller irregularly distributed tubercles on head, eyes, and dorsum. Ventral

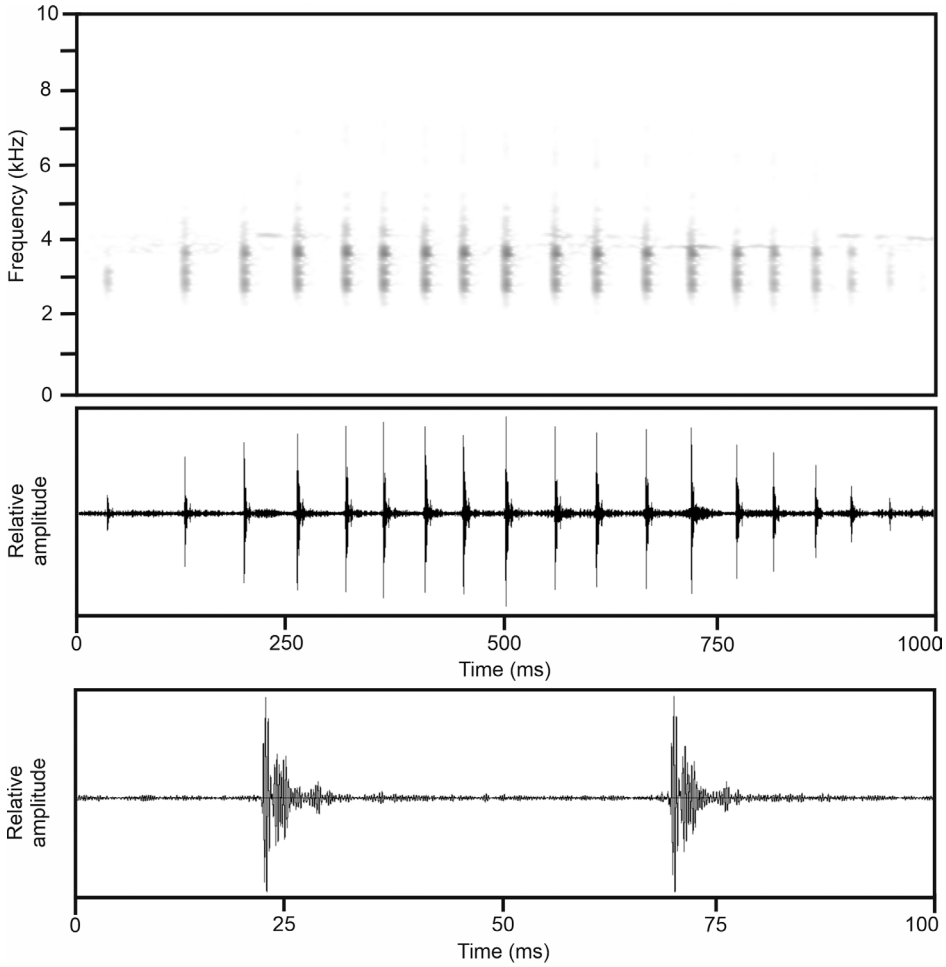

Figure 21. Audiospectrogram and corresponding oscillogram of one advertisement call of *Gephyromantis fiharimpe* **sp. nov.** recorded on 18 February 1994 at Ankeniheny. The oscillogram below shows a 100 ms section of the call figured above, showing two notes and their repective amplitude structure. Recording band-pass filtered at 1500–8000 Hz.

skin smooth on throat, chest and limbs, slightly granular on posterior belly. Femoral glands well delimited and distinctly recognizable from external view, apparently with two large gland granules in external view. Tibial glands distinct, covering about two thirds of the shank.

After five years in preservative, dorsal coloration of head and body uniformly dark brown, with darker crossbands on hind- and forelimbs. Posterodorsal surface of thigh largely pigmentless whitish/cream: this area in life was presumably reddish. Ventrally, throat, chest and anterior belly uniformly blackish brown, posteror belly fading into gray-cream. Ventral side of hindlimbs pigmentless cream, probably corresponding to reddish color in life.

Variation. A tibial gland is visible in all examined adult specimens, as well as in additional photographed individuals (UADBA-CRH 119, UADBA-CRH 486, UADBA-CRH 510, KU 340759 [CRH 511], KU 340736 [CRH 470]). The specimen ZMA 19421 from Ranomafana is probably a female (sex cannot be unambiguously determined due to removal of inner organs and part of the skin for chromosome analysis); this specimen also appears to have a tibial gland, which however cannot be recognized with full reliability. However, a second female (ZFMK 57435), which is assigned to this species tentatively (due to the lack of genetic data), has distinct tibial glands. The two females are larger than the males (25.7–25.8 vs. 22.0–24.0 mm SVL). For morphometric measurements of ZFMK and NMBE paratypes not included in Table 1, see Vences et al. (2002).

Call. The advertisement call (Fig. 21) was recorded on 18 February 1994 at Ankeniheny (air temperature 23.5°C; Vences et al. 2006: CD2, track 28). It consists of a multinote call of variable duration, emitted in series at regular intervals. Slight amplitude modulation is recognizable within calls, with notes at the beginning and the end of the call having lower call energy. Notes are very short and appear to consist of a single pulse each. They are repeated at irregular intervals within calls. Numerical call parameters of 12 analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 609–935 ms (798.8 ± 110.8 ms); note duration 7–9 ms (7.8 ± 0.8 ms); number of notes per call 11–19 (15.5 ± 2.4); note repetition rate within calls 14.9–25.6 notes/second (18.3 ± 3.9 notes/second); call repetition rate within call series approximately 24–25 calls/minute; dominant frequency 3638–3723 Hz (3676 ± 23 Hz); prevalent bandwidth 2500–5200 Hz.

Additional calls recorded on 1 January 1994 at Andasibe (temperature unknown) generally agree in characteristics with those reported from Ankeniheny, including evident variation in note repetition rate within calls. The main differences compared to calls from Ankeniheny are longer call duration and higher number of notes per call. Numerical call parameters of 7 analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 840–1503 ms (1179.3 ± 232.7 ms); note duration 5–12 ms (7.6 ± 2.2 ms); number of notes per call 26–43 (36.9 ± 7.3); note repetition rate within calls 18.2–37.4 notes/second (25.5 ± 6.5 notes/second); call repetition rate within call series approximately 25–27 calls/minute; dominant frequency 3649–4078 Hz (3796 ± 157 Hz); prevalent bandwidth 2500–4800 Hz.

Calls recorded on 12 January 2015 at Vohidrazana (air temperature 18.5°C; call voucher KU 340736 [CRH 470]) also agree with those from Andasibe in all general characters, being only slightly longer in duration on average and exhibiting slightly shorter note duration. Irregular note repetition rate within calls is evident. Numerical call parameters of 12 analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 950–1764 ms (1405.6 ± 290.4 ms); note duration 2–7 ms (4.3 ± 1.7 ms); number of notes per call 26–52 (40.7 ± 11.5); note repetition rate within calls 18.8–54.1 notes/second (32.1 ± 13.1 notes/second); call repetition rate within call series approximately 21–24 calls/minute; dominant frequency 3726–3913 Hz (3781 ± 68 Hz); prevalent bandwidth 2300–5000 Hz.

Calls recorded on 13 February 2015 at Ambatolahy, Ranomafana National Park (air temperature 17.6°C; call voucher KU 340759 [CRH 511]), based on genetic data assignable to clade B are very similar to the calls described for clade B from Andasibe, Ankeniheny, and Vohidrazana, but differ from these by a regular note repetition rate within calls and pronounced amplitude modulation within calls, with call energy continuously increasing from the beginning, reaching its maximum at the middle of the call, then continuously decreasing towards its end. Numerical call parameters of 14 analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 916–1162 ms (1073.9 ± 86.0 ms); note duration 4–7 ms (6.2 ± 0.9 ms); number of notes per call 27–34 (30.6 ± 2.3); note repetition rate within calls 25.6–28.0 notes/second (26.8 ± 1.0 notes/second); call repetition rate within call series approximately 20–23 calls/minute; dominant frequency 3305–3736 Hz (3523 ± 145 Hz); prevalent bandwidth 2100–5000 Hz.

Distribution and natural history. Based on genetically verified records, the distribution area includes in a south-north direction the localities (1) Vevembe, (2) Ranomafana, (3) Mandraka, (4) Maromizaha, (5) Andasibe, (6) Ambatovy, (7) Vohidrazana, (8) Anjozorobe, and (9) Ambohitantely. Probably the species is also present at Ankeniheny. The known elevational range of the species spans from 580 m a.s.l. (Vevembe) to approximately 1500 m a.s.l. (Ambohitantely). The species apparently is restricted to rather intact rainforest habitat. Males call at night from perch heights of 5–50 cm in the low understory vegetation, not concentrated around water bodies.

Gephyromantis oelkrugi sp. nov. (lineage C)

http://zoobank.org/8795A6C9-37D1-439C-A396-0E7FD-D8A02CF

Holotype. ZSM 314/2010 (FGZC 4220), adult male (call voucher), from Ambodivoangy, near Makira Reserve (15.2899°S, 049.6203°E, ca. 100 m a.s.l.), Analanjirofo Region, northeastern Madagascar, collected on 31 March 2010 by F. Glaw, J. Köhler, P.-S. Gehring, M. Pabijan, and F.M. Ratsoavina.

Paratypes. ZSM 315/2010 (no field number), adult male, with same collection data as holotype except for being collected on 02 April 2010; ZSM 271/2016 (FGZC 5300), probably an adult female, from Masoala Peninsula, near "Eco-Lodge chez Arol" hotel (ca. 15.7122°S, 49.9640°E, 21 m a.s.l.), Analanjirofo Region, northeastern Madagascar, collected on 09 August 2016 by F. Glaw, D. Prötzel, J. Forster, K. Glaw, and T. Glaw; MRSN A5475 (no field number), adult female, from an unspecified locality in the Masoala region, date unspecified, collected by J.E. Randrianirina; MRSN A1991 (FN 7905) and MRSN A1992 (FN 7910), adult individuals (unsexed), from Masoala Peninsula near Andasin'i Governera (campsite Ambatoledama ca. 15.2833°S, 50.0208°E), around the border between Analanjirofo and Sava Regions, northeastern Madagascar, collected on 09 December 1998 by F. Andreone and J.E. Randrianirina; MRSN A2844 (RJS 528), adult individual (unsexed), from Masoala Peninsula, near Mahalevona (campsite Amparihy, ca. 15.4177°S, 49.9403°E, ca. 770 m a.s.l.), Analanjirofo Region, northeastern Madagascar, collected on 09 February 2002 by J.E. Randrianirina; UADBA uncatalogued (FGZC 4201 and 4244), two adult specimens from the type locality Ambodivoangy collected on 31 March and 2 April 2010 by the same collectors as holotype; UADBA uncatalogued (APR 11885), from Sahabe Antanamahalana (15.5604°S, 050.2818°E, 45 m a.s.l.), Masoala National Park, Sava Region, northeastern Madagascar, collected in 2015 by A.P. Raselimanana; UADBA uncatalogued (APR 12006), from Ambohitsitondroina (15.5694°S, 050.0034°E, 550 m a.s.l.), Masoala National Park, Analanjirofo Region, northeastern Madagascar, collected in 2015 by A.P. Raselimanana.

Etymology. The specific epithet is a patronym for Christopher Roland Oelkrug in recognition of his support for biodiversity research and nature conservation through the BIOPAT initiative.

Diagnosis. A member of the subfamily Mantellinae based on the presence of intercalary elements between terminal and subterminal phalanges of fingers and toes (verified externally), and on the absence of nuptial pads and pres-

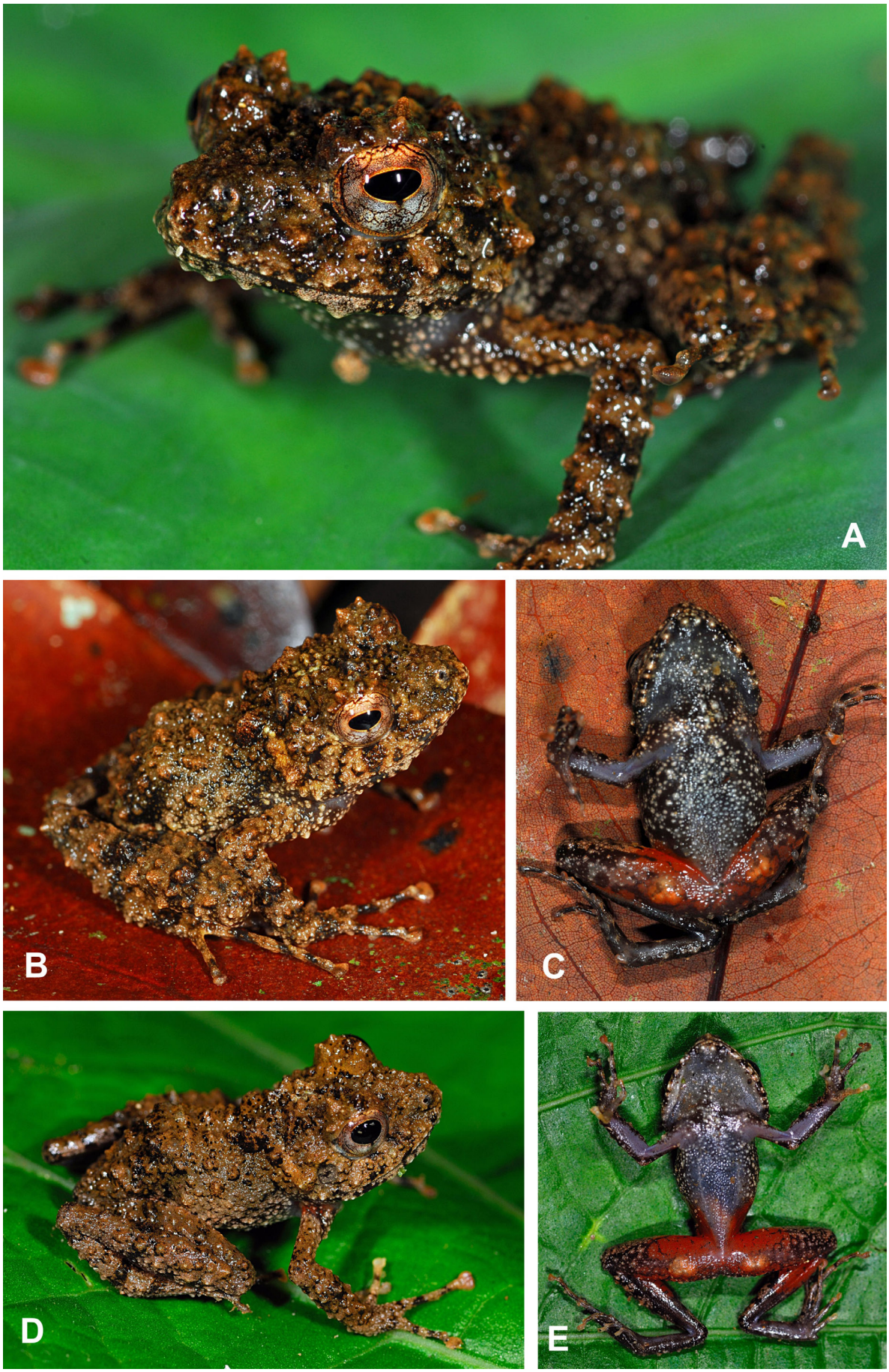

Figure 22. Specimens of *Gephyromantis oelkrugi* **sp. nov.** (lineage C) from the type locality, Ambodivoangy, in life. **A**, **B**, **C** Adult male (FGZC 4201) in frontal, dorsolateral and ventral view. **D**, **E** Adult male holotype (ZSM 314/2010, field number FGZC 4220) in dorsolateral and ventral view. Not to scale.

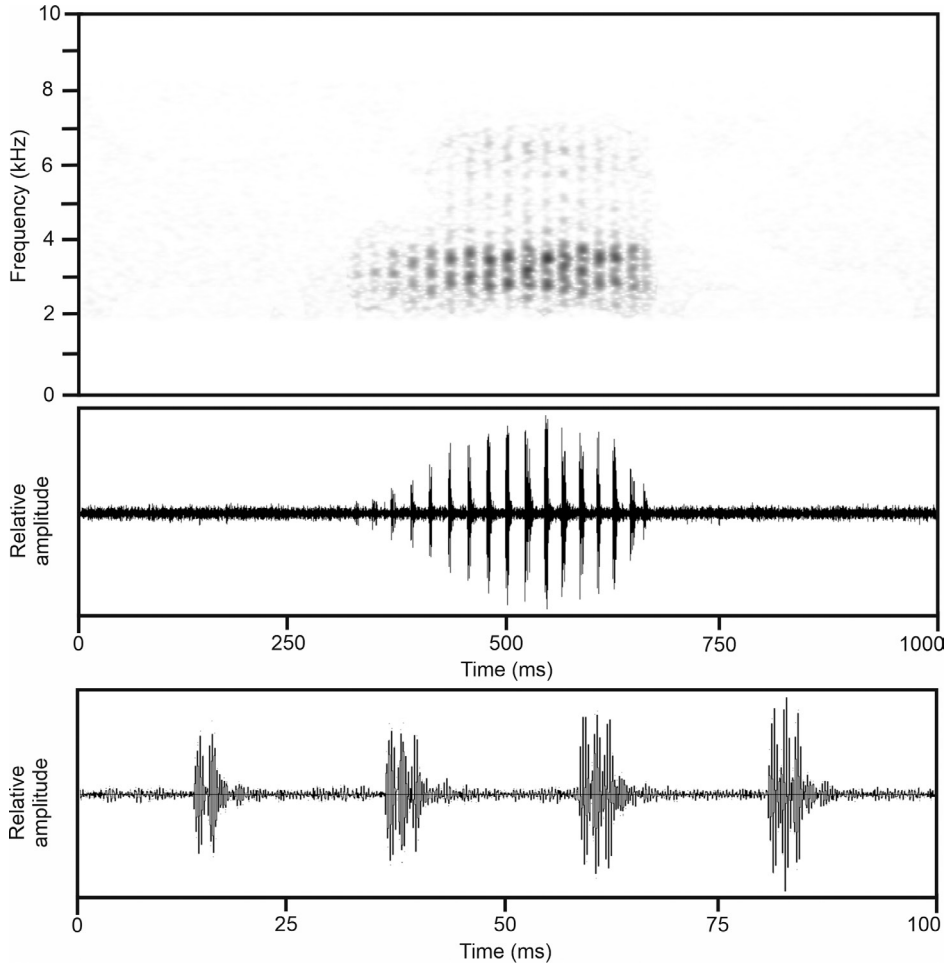

Figure 23. Audiospectrogram and corresponding oscillogram of one advertisement call of the holotype of *Gephyromantis oelkrugi* **sp. nov.** (ZSM 314/2010) recorded on 31 March 2010 at Ambodivoangy. The oscillogram below shows a 100 ms section of the call figured above, showing four notes and their repective pulse structure. Recording band-pass filtered at 2000–8000 Hz.

ence of femoral glands in males. Assigned to the subgenus *Laurentomantis* in the genus *Gephyromantis* based on the strongly tubercular dorsal skin, absence of foot webbing, completely connected lateral metatarsalia, and molecular phylogenetic relationships. The new species differs from all nominal species of the subgenus *Laurentomantis* as follows: From *G. horridus* by smaller body size (male SVL 21.6–21.9 mm vs. 33.5 mm), and absence of a dorsal pattern of two blackish transverse patches (vs. presence); from *G. ranjomavo* by the absence of light brown to orange-brown color covering limbs dorsally (vs. presence), a slightly smaller body size (male SVL 21.6–21.9 mm vs. 23.5–28.1 mm) and absence of a tibial gland (vs. presence); from *G. striatus* by absence of a vertebral stripe posteriorly on dorsum (vs. presence); from *G. malagasius* (as redefined herein) by absence of a distinct bluish gray pattern on a dark venter (vs. presence); from *G. marokoroko* by a more coarsely tubercular dorsal skin, presence of red color ventrally on limbs (vs. absence), absence of orange spots and vermiculations on dorsum (vs. presence), and absence of gray to whitish color on vocal sac (vs. presence). Furthermore, differing from all the aforementioned species by the presence of bright red color in the inguinal region and ventrally on limbs and on a small portion of posterior belly in life (vs. absence), and by a substantial genetic divergence (>6% uncorrected pairwise distance in the 16S gene).

According to the molecular phylogeny, *G. oelkrugi* is closely related to *G. fiharimpe* and *G. matsilo* described above. It differs from *G. fiharimpe* by the absence of a tibial gland (vs. presence), a more strongly tubercular dorsal skin, and brighter red ventral color that appears not to extend much on posterior belly (vs. less bright light red color extending onto posterior belly), probably by a faster note repetition rate in advertisement calls (43.5–54.1 vs. 14.9–37.4 notes/second in most recordings; but up to 54.1 in one recording of *G. fiharimpe*), and an uncorrected 16S genetic distance of 4.3–5.5%. The new species is morphologically most similar to *G. matsilo* but differs by a faster note repetition rate (43.5–54.1 vs. 20.8–21.6 notes/second) and a shorter note duration (3–10 ms vs. 13–21 ms) in advertisement calls, and an uncorrected 16S genetic distance of 4.3–5.5%.

For a distinction from the fourth new species described in the following (lineage D), see the diagnosis in the respective species account below.

Description of the holotype. Adult male in good state of preservation (Fig. 18), tongue removed as tissue sample for molecular analysis. SVL 22.0 mm, for other measure-

ments see Table 1. Body slender; head as wide as long, and as wide as body; snout rounded in dorsal view, truncate in lateral view; nostrils directed laterally, slightly protuberant, much nearer to tip of snout than to eye; canthus rostralis rather indistinct but strongly concave; loreal region slightly concave; tympanum distinct, rounded, 50% of eye diameter; no supratympanic fold; tongue removed and thus not available for examination; vomerine teeth absent; choanae rounded; maxillary teeth present. Dermal fold along the posterior part of the lower jaws (the inflatable parts of the vocal sac) not recognizable. Arms slender, subarticular tubercles single; outer and inner metacarpal tubercles moderately expressed, well recognizable; fingers without webbing; relative length of fingers 1 < 2 < 4 < 3, second finger distinctly shorter than fourth finger; finger discs enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaching between eye and nostril when hindlimb is adpressed along body; lateral metatarsalia connected; inner metatarsal tubercle distinct, outer metatarsal tubercle small but recognizable; webbing between fingers and toes absent; relative toe length 1 < 2 < 5 < 3 < 4. Third toe slightly longer than fifth toe. Toe discs enlarged. Skin on upper surface strongly granular but without distinct ridges; many smaller irregularly distributed tubercles on head, eyes and dorsum. Ventral skin smooth on throat, chest and limbs, slightly granular on posterior belly. Femoral glands well delimited and distinctly recognizable from external view. No tibial glands.

After eleven years in preservative, dorsal coloration of head and body uniformly dark brown, with darker crossbands on hind- and forelimbs. Posterodorsal surface of thigh with a large pigmentless patch in its distal part, this area presumably corresponding to reddish color in life. Ventrally, whitish, with some dark spotting along the lower lip, two symmetrical brown patches on chest, and brown pigment on distal part of thighs.

Variation. The coloration in life of several specimens is shown in Fig. 22. The examined specimens of *G. oelkrugi* as well as the additional specimens photographed are morphologically quite similar to each other. The two measured males agree in body size (SVL 21.6–21.9 mm; Table 1). The only known female is slightly smaller (SVL 21.1 mm) but has no oocytes visible through the belly skin, and we hypothesize it is rather a subadult or young adult. The femoral glands of the males appear to consist of 3–5 granules arranged in a somewhat circular manner around a central depression (Fig. 22C, E).

Call. The advertisement call (Fig. 23) was recorded on 31 March 2010 at Ambodivoangy (estimated air temperature 25°C) from the holotype (ZSM 314/2010, FGZC 4220). It consists of a series of very short pulsed notes. There is considerable amplidude modulation within each call, with call energy constantly increasing from the beginning of the call reaching the maximum amplitude at about 60% of its duration and from there decreasing towards its end. Within calls, notes are repeated at a high rate and at regular intervals. Numerical parameters of 10 analyzed calls are as follows (range followed by mean ± standard deviation in parentheses): call duration 279–504 ms (350.9 ± 88.4 ms); note duration 3–10 ms (6.8 ± 1.3 ms); number of notes per call 13–25 (16.9 ± 4.4); note repetition rate within calls 43.5–54.1 notes/second (46.5 ± 4.0 notes/second); pulses per note 1–4 (2.9 ± 0.7); dominant frequency 3266–3882 Hz (3539 ± 190 Hz); prevalent bandwidth 2100–4500 Hz. Calls were usually emitted isolated at rather irregular intervals, except for one short call series recorded. This series contained 3 calls repeated at a rate of approximately 34 calls/minute.

Distribution and natural history. Based on genetically verified records, the distribution area is restricted to (1) Ambodivoangy close to the Makira Reserve, and the Masoala Peninsula where it has been found at various sites, including (2) Andasin'i Governera, (3) Andranobe, (4) Ambatoledama, (5) Amparihy, (6) Antanamahalana, (7) Ambohitsitondroina, and (8) near hotel "Eco-Lodge chez Arol". A species apparently restricted to lowland rainforest habitat. The holotype was found calling sitting on a twig of a shrub plant approximately 30 cm above the ground.

Gephyromantis portonae sp. nov. (lineage D)

http://zoobank.org/BEDD6FC6-6F8C-4AE7-9F73-E41DF-76D4DA6

Holotype. ZSM 115/2021 (ACZCV 0032), adult male, from Sahaïndrana campsite (17.8971°S, 049.1991°E, ca. 239 m a.s.l.), Betampona Strict Nature Reserve, Antsinanana Region, eastern Madagascar, collected on 6 November 2013 by A. Crottini, D. Salvi, E. Scanarini, and J.H. Velo.

Paratypes. ZSM 118/2021 (ACZCV 0223), adult female, from Vohitsivalana campsite (17.8826°S, 049.2056°E, ca. 497 m a.s.l.), Betampona Strict Nature Reserve, Antsinanana Region, eastern Madagascar, collected on 16 November 2013 by A. Crottini, D. Salvi, E. Scanarini, and Georges; ZSM 116/2021 (ACZCV 0030), adult female, from Sahaïndrana campsite (17.8961° S, 049.1995° E, ca. 240 m a.s.l.), Betampona Strict Nature Reserve, collected on 6 November 2013 by A. Crottini, D. Salvi, E. Scanarini, and J.H. Velo; ZSM 117/2021 (ACZCV 0025), adult male, from Sahaïndrana campsite (17.8945° S, 049.1988° E, ca. 349 m a.s.l.), Betampona Strict Nature Reserve, collected on 6 November 2013 by A. Crottini, D. Salvi, E. Scanarini, and J.H. Velo; MRSN A6263 (FAZC 13518), adult female, from Sahambendrana campsite (17.8984°S, 049.2154°E, ca. 429 m a.s.l.), Betampona Strict Nature Reserve, collected on 7 February 2007 by G. M. Rosa, and F. Andreone; MRSN A6319 (FAZC 13469), adult male, from Piste Fontsimavo (17.9264°S, 049.2083°E, ca. 220 m a.s.l.), Betampona Strict Nature Reserve, collected on 3 February 2007 by G.M. Rosa; UADBA uncatalogued (ACZCV 0069), adult individual (unsexed), from Piste Fontsimavo (17.9186°S, 049.2103°E, ca. 256 m a.s.l.), Betampona Strict Nature Reserve, collected on

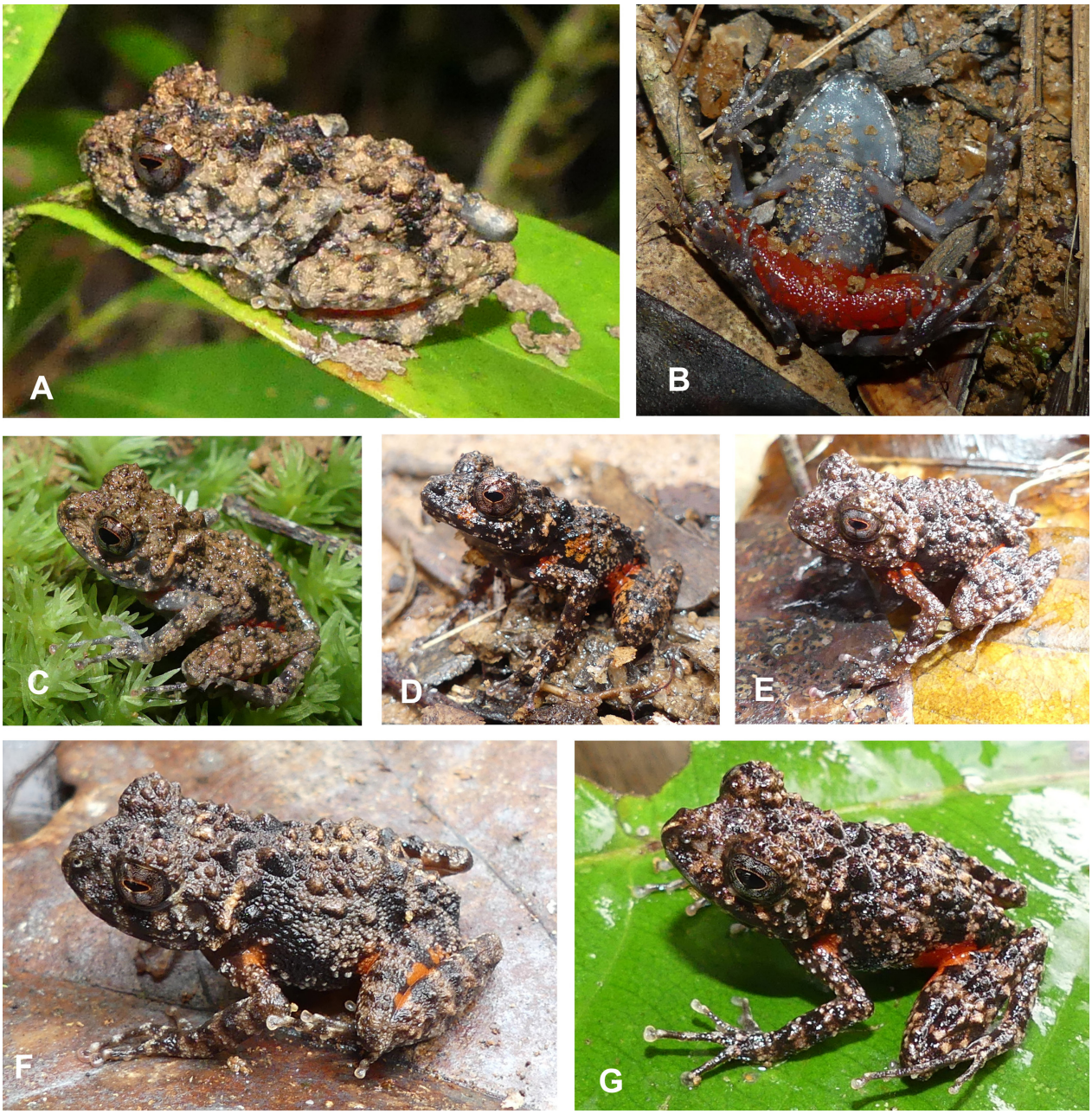

Figure 24. Specimens of *Gephyromantis portonae* **sp. nov.** (lineage D) in life, all from Betampona. **A**, **B** Specimen ACZCV 1216. **C** Specimen ACZCV 1215. **D** Specimen ACZCV 1311. **E** Specimen ACZCV 1353. **F** Specimen ACZCV 1350. **G** Specimen ACZCV 1358. Not to scale.

11 November 2013 by A. Crottini, D. Salvi, E. Scanarini, Georges, G.M. Rosa, D.J. Harris, M. Randriamialisoa, and H. Lava; UADBA uncatalogued (ACZCV 0031), juvenile, from Sahaïndrana campsite, Betampona Strict Nature Reserve, collected on 7 November 2013 by A. Crottini, D. Salvi, E. Scanarini, and J.H. Velo; UADBA uncatalogued (ACZCV 0024), adult individual (unsexed), from Sahaïndrana campsite, Betampona Strict Nature Reserve, collected on 5 November 2013 by A. Crottini, D. Salvi, E. Scanarini, and J.H. Velo.

Etymology. The specific epithet is a matronym for Ingrid Porton, our dear friend and colleague. Ingrid is a primatologist and Vice-Chair of Madagascar Fauna and Flora Group, and this honor is a recognition of her continuous support to the study of the unique biodiversity of Betampona Strict Natural Reserve, and her overall commitment to the conservation of Malagasy ecosystems.

Diagnosis. A member of the subfamily Mantellinae based on the presence of intercalary elements between terminal and subterminal phalanges of fingers and toes (verified externally), and on the absence of nuptial pads and presence of femoral glands in males. Assigned to the subgenus *Laurentomantis* in the genus *Gephyromantis* based on the strongly tubercular dorsal skin, absence of foot webbing, completely connected lateral metatarsalia, and molecular phylogenetic relationships. The new species differs from all nominal species of the subgenus *Laurentomantis* as follows: From *G. horridus* by smaller

body size (male SVL 22.4–22.9 mm vs. 33.5 mm), and absence of a dorsal pattern of two blackish transverse patches (vs. presence); from *G. ranjomavo* by the absence of light brown to orange-brown color covering limbs dorsally (vs. presence), a slightly smaller body size (male SVL 22.4–22.9 mm vs. 23.5–28.1 mm) and absence of a tibial gland (vs. presence); from *G. striatus* by absence of a vertebral stripe posteriorly on dorsum (vs. presence); from *G. malagasius* (as redefined herein) by absence of a distinct bluish gray pattern on a dark venter (vs. presence); from *G. marokoroko* by a more coarsely tubercular dorsal skin, presence of red color ventrally on limbs (vs. absence), absence of orange spots and vermiculations on dorsum (vs. presence), and absence of gray to whitish color on vocal sac (vs. presence). Furthermore, differing from all the aforementioned species (with the exeption of *G. fiharimpe*) by the presence of bright red color in the inguinal region and ventrally on limbs and on a small portion of posterior belly in life (vs. absence), and by a substantial genetic divergence (>6% uncorrected pairwise distance in the 16S gene).

According to the molecular phylogeny, *G. portonae* **sp. nov.** is related to *G. fiharimpe*, *G. matsilo*, and *G. oelkrugi* described above, but appears to be the genetically most divergent species of this complex, possibly representing the sister taxon of a clade composed by the other three species. It differs from *G. fiharimpe* by the absence of a tibial gland (vs. presence), a more strongly tubercular dorsal skin, and brighter red ventral color (vs. less bright light red color), and an uncorrected 16S genetic distance of 6.1–8.1%; from the sympatric *G. matsilo* by third toe distinctly longer than fifth (vs. of similar length or slightly longer) and an uncorrected 16S genetic distance of 5.9–7.3%; and from *G. oelkrugi* by a dorsal skin composed of mostly rather large and rounded tubercles (vs. equally prominent but smaller and more pointed tubercles), and an uncorrected 16S genetic distance of 5.1–7.5%.

Description of the holotype. Adult male in good state of preservation (Fig. 18), skin around left femoral gland cut for internal examination of gland. SVL 22.9 mm, for other measurements see Table 1. Body slender; head longer than wide, as wide as body; snout rounded in dorsal view, subacuminate in lateral view; nostrils directed laterally, slightly protuberant, much nearer to tip of snout than to eye; canthus rostralis rather indistinct, slightly concave; loreal region slightly concave; tympanum distinct, rounded, 50% of eye diameter; supratympanic fold not recognizable; tongue ovoid, distinctly forked posteriorly; vomerine teeth weakly recognizable, but present in two small aggregations posteromedially to choanae; choanae rounded; maxillary teeth present. Dermal fold along the lower jaws (the inflatable parts of the vocal sac) not recognizable. Arms slender, subarticular tubercles single; outer and inner metacarpal tubercles distinct; fingers without webbing; relative length of fingers 1 < 2 < 4 < 3, second finger distinctly shorter than fourth finger; finger discs distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaching anterior corner of eye when hindlimb is adpressed along body; lateral metatarsalia connected; inner metatarsal tubercle distinct, outer metatarsal tubercle small but distinct; webbing between toes absent; relative toe length 1 < 2 < 5 < 3 < 4. Third toe distinctly longer than fifth toe. Toe discs enlarged. Skin on upper surface coarsely granular, with a few small ridges and numerous large tubercles on head, eyes, and dorsum. Ventral skin smooth on throat, chest and limbs, granular on posterior belly. Femoral glands well delimited and large, with seven large gland granules visible both in external and internal view. No tibial gland.

After eight years in preservative, dorsal coloration of head and body dark brown, washed with lighter brown color especially on the large tubercles. Darker brown crossbands on hind- and forelimbs. Ventral and posterodorsal surface of thigh with larger whitish/cream areas, which were presumably reddish in life. Ventrally, throat light brown to grayish with a few small light spots; chest brown; belly marbled with brown and light color.

Variation. The specimens examined and photographed all agree well with each other in external morphology. Several photographed individuals have very large and coarse dorsal tubercles, of less spiny appearance compared to *G. oelkrugi* and *G. matsilo* (see especially Fig. 24A, but also 24E–G). Tibial glands are absent in all specimens. The two females are distinctly larger than the two males for which measurements are available (25.1–26.1 vs. 22.4–22.9).

Call. Although the advertisement call has never been recorded and analysed, one male has been heard calling. Call sounded unmotivated with low amplitude notes.

Distribution and natural history. Based on genetically verified records, the distribution area is restricted to (1) Sahafina and (2) Betampona. Calling males were observed in Betampona in a muddy bank close to slow running brooks, but the species is often encountered on the leaflitter in dense forest, along slopes and ridges of steep hills, far from water. The tadpole is unknown. A poorly known species apparently restricted to lowland rainforest habitat ranging from 200 m a.s.l. to approximately 500 m a.s.l. of elevation.

Discussion

Frogs of the subgenus *Laurentomantis* are among the least known anurans in Madagascar. For instance, their reproductive biology is almost completely unknown: no report of their mating behavior has been published, no clutches have been found, and only for one species (*G. malagasius*, under the name *G. ventrimaculatus*) have tadpoles been described (Randrianiaina et al. 2011). The species diversity of this group remains insufficiently known; however, we provided molecular, morphological and bioacoustic evidence for four new species. Delimit-

ing the four new species named herein has been difficult for many years due to the insufficient amount of data, and was only possible by integrating samples, specimens, and data collected by various research teams over almost three decades. The current picture suggests that *Laurentomantis* includes species that are widespread in the eastern rainforest belt, with limited molecular and morphological variation, such as *G. fiharimpe*, which occurs at mid-elevation localities (580–1500 m a.s.l.) from Vevembe to Anjozorobe and Ambohitantely (about 500 km linear distance), and is characterized across its range by a tibial gland lacking in its closest relatives. Also *G. matsilo* occurs at localities almost 600 km apart (Ambohitsara to Befanjana) but apparently at lower elevations (ca. 50–300 m a.s.l.), a pattern found in other rainforest frogs in Madagascar (e.g., *Aglyptodactylus*; Köhler et al. 2015). Other species such as *G. oelkrugi* and *G. portonae* have more restricted ranges, conforming to an overall high rate of microendemism in Madagascar (e.g., Köhler et al. 2010; Brown et al. 2016; Rakotoarison et al. 2017).

Although we assigned all known samples and specimens of *Laurentomantis* to species, several taxonomic enigmas still require revision. This mainly concerns *G. horridus* and *G. ranjomavo* where the populations assigned to *G. ranjomavo* present a substantial genetic divergence, possibly indicating a species complex (additional material and bioacoustic data from across the range of *G. ranjomavo* are needed to clarify this question). Conversely, since no fresh collections (and thus no male specimens and no molecular data) of *G. horridus* from its type locality Nosy Be are available, the identity of this species remains uncertain. Based on morphological similarity, we have followed previous studies (e.g., Vences et al. 2002; Glaw and Vences 2011) considering the population from Montagne d'Ambre as *G. horridus.* This is plausible as other species also occur both at Nosy Be and Montagne d'Ambre with relatively low genetic divergences (e.g., *G. granulatus*; data in Vences et al. 2003). However, in other cases, the two sites harbor closely related but distinct species, such as *Mantidactylus bellyi* vs. *M. ulcerosus*. Only additional collections from Nosy Be, or DNA barcode fishing from the holotype of *G. horridus* (which we did not attempt for the present study) can provide a conclusive answer to the identity of *G. horridus*.

The molecular allocation of the holotype of *Microphryne malagasia* to the lineage previously considered as *G. ventrimaculatus* is perhaps the most suprising result obtained by DNA barcode fishing in historical types of Malagasy anurans (see: Rancilhac et al. 2020; Scherz et al. 2020). The specimen lacks the typical color pattern known from fresh material of this lineage, has a substantially smaller body size than the smallest other adult individual known, and comes from a site where the lineage was previously unknown (Folohy; see Fig. 2). For instance, despite intensive survey work at Betampona, a reserve close to the assumed geographical position of the *malagasius* type locality Folohy, no specimens of this lineage have been found (e.g., Rosa et al. 2012). However, after carefully evaluating the molecular evidence, we are convinced our results cannot be explained by sample confusion, contamination or artefacts. The sample of the holotype was taken by one of us (LdP) in the Transvaal Museum in Pretoria, with instruments and vials coming from a lab where no *Laurentomantis* DNA or tissue had been processed before. The TMP collection to our knowledge does not contain other *Laurentomantis*, which could have shed DNA into the preservative. The tissue sample was then transferred to a laboratory (Potsdam University) specialized in ancient DNA study where no *Laurentomantis* sample has ever been processed to date. The phylogenetic placement of the sequence obtained by these means is totally unambiguous (Fig. 1). Furthermore, the allocation is supported by SNP positions across the 16S gene (Fig. S1). Fully diagnostic SNPs were found on at least three stretches of the gene that correspond to separate gene fragments each captured by a different bait during the target-capture procedure. This increases the probability that all these DNA fragments originated from the same biological organism and do not represent contamination or sequencing errors. In addition, the confirmation of the presence of this lineage in Andasibe, at an estimated 100–150 km distance from the historical locality Folohy, confirms it as a widespread lineage: the linear distance between the extreme southeastern site Isaka-Ivondro to Andasibe spans more than 650 km. Andasibe is one of the best studied sites in Madagascar (Colwell and Lees 2000; Vieites et al. 2009; Brown et al. 2016) and the fact that only three observations of this species have so far been made here makes it likely that it has also been overlooked elsewhere. Still, this example also serves to illustrate that great caution is to be taken when using results from archival DNA analysis to resolve an important extrinsic hindrance to taxonomic progress – clarifying the identity of historical type material (Scherz et al. 2020). In particular, given the risks of contamination and of destabilization of established names by premature conclusions, archival DNA results always need to be critically evaluated as they are not free of remaining uncertainties. In our case, the available evidence allows us to exclude possible contamination with sufficient reliability, and we here therefore used the molecular data to overrule the indications from morphology (body size, coloration, femoral gland structure), which would not immediately favor an assignment of the holotype to the lineage previously considered as *G. ventrimaculatus*.

According to the expanded data presented in this study, only two species of *Laurentomantis* have a distinct tibial gland: *G. fiharimpe* and *G. ranjomavo*. Given that for all species in the subgenus we have examined males emitting advertisement calls in the rainy season (thus sexually mature and reproductively active), and the presence or absence of these males could unambiguously be ascertained in all of them, it is obvious that absence of glands is not strictly linked to seasonal or ontogenetic effects. Considering the scarcity of material for some species, we cannot fully exclude individual, sexual, geographical or seasonal variation of this character, but it seems clear that it is genuinely absent in most species. Although our phylogenetic tree (Fig. 1) is based on only a single mitochondrial gene, there is overwhelming evidence for independent evolu-

tion of this character state in the two species in which it has been ascertained. First, the two species are not each other's closest relatives: *G. ranjomavo* is with high support sister to *G. horridus*, which does not have a tibial gland according to the new data presented herein; and *G. fiharimpe* (with gland) is sister to *G. matsilo* (lacking a gland). These relationships were also recovered by Kaffenberger et al. (2012) in a multi-gene data set, with full node support. Second, a tibial gland is not found in other *Gephyromantis* and therefore most probably is a derived state. As already discussed by Glaw and Vences (2011), males of all members of the subfamily Mantellinae in the family Mantellidae, to which *Gephyromantis* belongs, are characterized by femoral glands (Glaw and Vences 2006), and in addition, males of some species of *Gephyromantis* (subgenera *Asperomantis* and *Duboimantis*) have a unique protuberance at the base of the forelimb that may represent a humeral gland (Vences and Glaw 2001, Vences et al. 2017). However, besides the two species of *Laurentomantis*, tibial glands are only found in one other species of Malagasy frog (the microhylid *Rhombophryne guentherpetersi*; Glaw and Vences 2011). They thus seem to represent repeatedly derived novel structures. Their function is therefore of great interest.

Despite a substantially improved understanding of the diversity of *Laurentomantis*, some species of this subgenus continue to be among the least collected frogs in Madagascar. For example, the recently described *G. marokoroko* (Hutter et al. 2022) went unnoticed for decades despite intensive fieldwork of multiple teams at its type locality, Vohidrazana, and the morphologically very distinct *G. malagasius* (as redefined herein) is still only known from three observations in the intensively surveyed Andasibe region. Certainly, the secretiveness of these frogs and their inconspicuous low-intensity calls, are added difficulties to locate them in the field. Yet, we hypothesize that some *Laurentomantis* may indeed occur at low densities or be locally restricted to certain habitat patches. Although the widespread occurrence of some *Laurentomantis* across many forests and protected areas in eastern Madagascar characterizes them as potentially Least Concern species in terms of the IUCN Red List (IUCN 2001), it is important to highlight that assessing population trends for such low-density species is inherently difficult. Any silent declines without mass mortality, e.g. driven by pathogens such as chytrid fungi (Bletz et al. 2015) will be difficult to detect for these frogs. As a consequence, we highlight the importance of reporting any observation of *Laurentomantis* (and other rare species) via portals such as iNaturalist, and to continue with regular intensive survey and monitoring work in selected forest sites in Madagascar, even in those with a supposedly rather complete amphibian inventory.

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Competing interests

The authors have declared that no competing interests exist.

Author contributions

MV: Conceptualization, Investigation, Writing - Original draft. JK, Investigation, Writing – Original draft. FG, MDS, AC: Resources, Data Curation, Writing – Review and Editing. MP, MH, Methodology, Investigation, Writing – Review and Editing. CRH, LdP, AR, APR, GMR, Resources, Writing – Review and Editing.

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Supplementary material 1

Table S1

Authors: Vences M, Köhler J, Crottini A, Hofreiter M, Hutter CR, Du Preez LH, Preick M, Rakotoarison A, Rancilhac L, Raselimanana A, Rosa GM, Scherz MDD, Glaw F (2022)

Data type: .docx

Explanation note: Summary of uncorrected pairwise distances between species in the subgenus *Laurentomantis*. For each pairwise between-species comparison, the minimum and maximum value from pairwise comparisons between individuals is reported. Cells marked in green report values of intra-specific distances (i.e., distances between individual sequences assigned to the same species, minimum and maximum). Yellow cells mark comparisons between lineages A–D, corresponding to the four new species described in this study.

Link: https://doi.org/10.3897/vz.72.e78830.suppl1

Supplementary material 2

Figure S1

Authors: Vences M, Köhler J, Crottini A, Hofreiter M, Hutter CR, Du Preez LH, Preick M, Rakotoarison A, Rancilhac L, Raselimanana A, Rosa GM, Scherz MDD, Glaw F (2022)

Data type: .jpeg

Explanation note: Sections of the assembly of Illumina reads obtained by targeted capture from DNA extracted from the holotype of *Microphryne malagasia* (TMP 10076), aligned to reference sequences (marked by blue rectangle) of the 16S rRNA gene of lineages of the subgenus *Laurentomantis* occurring along Madagascar's east coast. The yellow bars mark nucleotide position in which the respective reads agree with the reference sequence of the lineage previously considered as *Gephyromantis ventrimaculatus* but differ from at least one (but not all) of the other lineages. Red bars mark nucleotide sequences in which the reads agree with *G.* "*ventrimaculatus*" and differ from all other reference sequences included (fully diagnostic sites). Note that only a few reads are shown; many more reads were obtained for each captured section, and for the sections shown here these were all uniform. (Note: sp. 1, 2, 3, 4 in the labels refer to lineages A, B, C, D, respectively).

Link: https://doi.org/10.3897/vz.72.e78830.suppl2