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An initial molecular resolution of the mantellid frogs of the *Guibemantis liber* complex reveals three new species from northern Madagascar

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Abstract

The small arboreal frog *Guibemantis liber* (Anura: Mantellidae) has served as an example for the existence of deep conspecific lineages that differ by a substantial amount in mitochondrial DNA but are similar in morphology and bioacoustics and thus are assigned to the same nominal species. During fieldwork in northern Madagascar, we identified additional such lineages and surprisingly, observed close syntopy of two of these at various sites. In-depth study based on DNA sequences of the mitochondrial cytochrome *b* gene from 338 specimens of *G. liber* sensu lato from across its range, sequences of four nuclear-encoded markers for 154–257 of these specimens, a phylogenomic dataset obtained by the FrogCap target capture approach, and additional mitochondrial genes for representatives of most mitochondrial lineages, as well as bioacoustic and morphological comparisons, revealed concordant differentiation among several lineages of the *G. liber* complex. We identify nine lineages differing by 5.3–15.5% in cytochrome *b* and 2.4–10.1% in the 16S rRNA gene, and find that several of these lack or have only limited allele sharing in the nuclear-encoded genes. Based on sympatric or parapatric occurrence without genetic admixture, combined with differences in bioacoustic and morphological characters, we scientifically name three lineages from northern Madagascar as new species: *G. razoky* **sp. nov.**, *G. razandry* **sp. nov.**, and *G. fotsitenda* **sp. nov.** Of these new species, *G. razoky* **sp. nov.** and *G. razandry* **sp. nov.** show widespread syntopy across northern Madagascar and differ in body size and advertisement calls. *Guibemantis fotsitenda* **sp. nov.** is sister to

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G. razandry **sp. nov.**, but appears to occur at lower elevations, including in close geographic proximity on the Marojejy Massif. We also detected subtle differences in advertisement calls among various other mitochondrial lineages distributed in the Northern Central East and Southern Central East of Madagascar, but the status and nomenclatural identity of these lineages require further morphological and bioacoustic study of reliably genotyped individuals, and assignment of the three available names in the complex: *Rhacophorus liber* Peracca, 1893, *Gephyromantis albogularis* Guibé, 1947, and *Gephyromantis variabilis* Millot and Guibé, 1951. We discuss the identity and type material of these three nomina, designate a lectotype for *Gephyromantis variabilis* from Itremo, and flag the collection of new material from their type localities, Andrangoloaka and Itremo, as paramount for a comprehensive revision of the *G. liber* complex.

Keywords

Amphibia, Anura, bioacoustics, FrogCap, morphology, *Pandanusicola*, phylogeography, taxonomy

Introduction

Madagascar is renowned for the high proportion of microendemism of its biota (Wilmé et al. 2006; Vences et al. 2009), that is, many species occur in only very small ranges (Brown et al. 2016), often separated by considerable geographical distances from their closest relatives on the island. On the other hand, Madagascar also harbors numerous widespread species of varying degrees of phylogeographic structure, a phenomenon well studied in amphibians and reptiles. Some species of Madagascar's herpetofauna, such as the frogs *Laliostoma labrosum*, *Boophis albilabris* or the *Mantella baroni* complex, occur over hundreds of kilometers without substantial genetic differentiation of their populations (Rabemananjara et al. 2007; Pabijan et al. 2015; Glaw et al. 2018), whereas other amphibians and reptiles consist of deeply divergent phylogroups which sometimes have been named as distinct species, sometimes are considered as conspecific, and in other cases still require further study (e.g., Florio et al. 2012; Vences et al. 2014; Grbic et al. 2015; Rodríguez et al. 2015). Macroecological analyses have shown that small-sized species among Madagascar's herpetofauna in general have smaller ranges compared to large-sized species (Wollenberg et al. 2011; Brown et al. 2016), that small-sized species of frogs as well as those occurring in areas of high elevational heterogeneity are more prone to diverge genetically (Wollenberg et al. 2008; Pabijan et al. 2012), and that frogs specialized to forest habitat and/or from topographically complex regions show higher phylogeographic structure (Rodríguez et al. 2015).

The dichotomy between microendemic vs. widespread species also characterizes *Pandanusicola*, a subgenus in the mantellid genus *Guibemantis* (Glaw and Vences 2006). Of currently 18 species in *Guibemantis* (Amphibiaweb 2022), 13 belong to *Pandanusicola*. Most of these are specialized frogs that live and breed exclusively in the leaf axils of *Pandanus* screw pines; but at least two species (*Guibemantis liber* and *G. tasifotsy*) often use *Pandanus* leaf axils as shelter during the day but reproduce in open swamps and ponds (Lehtinen et al. 2007, 2011, 2012, 2018; Bletz et al. 2018). The *Pandanus*-reproducing species are characterized by a remarkable uniformity in their external morphology, and their advertisement calls are inconspicuous and poorly known; their distinction is mostly based on coloration and genetics, and as far as known most species have restricted ranges (Lehtinen et al. 2018). On the other hand, the two swamp-breeding species can be recognized by a set of morphological characters, in particular femoral gland morphology (Glaw et al. 2000; Vences et al. 2007), and have loud and distinct advertisement calls. One of these swamp-breeding species, *Guibemantis* (*Pandanusicola*) *liber* (Peracca, 1893), is, according to current taxonomy, the most widespread species of the subgenus *Pandanusicola*, and indeed of *Guibemantis* altogether, occurring from the extreme northern Montagne d'Ambre Massif southwards at least until the south-eastern coastal Manombo Reserve. It was the first species in which the unique amplexus-free mating behavior of mantelline frogs was documented (Blommers-Schlösser 1975). These frogs mate on leaves overhanging lentic water bodies such as swamps or shallow ponds (Blommers-Schlösser 1975). Their clutches of greenish eggs attach to these leaves and the tadpoles drop into the water where they complete their development. Tadpoles are exotrophic and generalized (Blommers-Schlösser 1975, 1979).

Across its range, *G. liber* is a rather common frog, characterized by a striking and confusing polymorphism in dorsal coloration which is exacerbated by the fact that males in the peak of the reproductive season become very dark, sometimes almost blackish, with a strongly contrasting bright white subgular vocal sac (Blommers-Schlösser 1975, 1979; Glaw and Vences 2007). The species is also known to be genetically variable: Vieites et al. (2009) used *G. liber* as an example of a frog species showing multiple deeply divergent genetic lineages across its range, which they interpreted as intraspecific variation. We here acknowledge this high variation identified in *G. liber* by referring to the assemblage of lineages as the *G. liber* complex.

In the present study we provide data on the phylogeography and systematics of the *G. liber* complex based on a comprehensive molecular sampling. We confirm vari-

ous deep mitochondrial lineages which appear to admix widely, whereas three other lineages appear to be genetically isolated, despite sympatry at numerous sites, and are here formally named as new species.

Materials and Methods

Sample collection and morphometric measurements

Samples for this study were collected during various field campaigns in Madagascar between 2000–2016. Frogs were caught either during nocturnal searches, typically by locating calling males and breeding individuals in swamps, or during the day by searching in *Pandanus* leaf axils and similar microhabitats. Frogs were anesthetized by immersion in MS222 or chlorobutanol solution and subsequently euthanized by overdose of the same substances. Tissue samples for molecular analysis were removed and stored separately in 1.5 ml vials with pure ethanol. Vouchers were then fixed in 95% ethanol, preserved in 70% ethanol. We deposited vouchers in various collections, primarily the Zoologische Staatssammlung München, Germany (ZSM), Zoological Museum Amsterdam, Netherlands (ZMA; collections now in Naturalis, Leiden), University of Kansas, Lawrence, USA (KU), and the Université d'Antananarivo, Département de Biologie Animale, Madagascar (UADBA). Additionally, type material was studied from the Museum National d'Histoire Naturelle, Paris, France (MNHN) and the Natural History Museum, London, UK (formerly BMNH, now NHMUK). The acronym MRSN refers to the Museo Regionale di Scienze Naturaly in Torino, Italy. FGZC, FGMV and ZCMV refer to field numbers of F. Glaw and M. Vences. MSZC, DRV, PSG, CRH, and MPFC refer to field numbers of M.D. Scherz, D.R. Vieites, P.-S. Gehring, C.R. Hutter, and M. Pabijan, respectively. Geographical regions within Madagascar are named according to Boumans et al. (2007).

Morphometric measurements were taken by TK and MV at an accuracy of 0.1 millimeter with a manual caliper. The following measurements were taken: snout–vent length (SVL); maximum head width (HW); head length from tip of snout to posterior edge of snout opening (HL); horizontal tympanum diameter (HTD); horizontal eye diameter (HED); 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.

Bioacoustics

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), and with a digital recorder with built-in microphones (Edirol R-09). 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) at Hanning window function. Spectrograms were drawn at Blackman window function with 256 bands resolution. In some cases, sensitive filtering was used 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. Terminology and methods in call analyses and descriptions follow the recommendations of Köhler et al. (2017), using the call-centered terminological scheme. Original call recordings in .wav format are available from the Zenodo repository (https://doi.org/10.5281/zenodo.7474341).

Molecular datasets

To examine the phylogeography and genetic differentiation within the *G. liber* complex, we assembled various molecular datasets:

(1) For an initial screening of genetic variation in all available samples, we used a fragment of the mitochondrial cytochrome *b* (COB) gene which has previously been used in assessments of genetic diversity in Madagascar frogs (Rodríguez et al. 2015). DNA was salt-extracted and the COB fragment amplified using the primer pair Cytb-a and Cytb-c of Bossuyt and Milinkovitch (2000).

(2) To understand the concordance between the variation in mitochondrial and nuclear-encoded genes, we amplified fragments of four nuclear-encoded, protein-coding genes: recombination-activating gene 1 (RAG1), brain-derived neurotrophic factor (BDNF), tyrosinase (TYR) and proopiomelanocortin (POMC). For primers used, see Table S1.

(3) To infer the phylogenetic position of lineages in the *G. liber* complex within the subgenus *Pandanusicola*, we assembled a multi-gene dataset for representative samples of the major lineages, and of all other species in the subgenus. This dataset consisted of the five genes in datasets 1 and 2 (COB, RAG1, BDNF, TYR, POMC) plus the nuclear gene recombination-activating gene 2 (RAG2) and the mitochondrial genes for 12S and 16S rRNA and cytochrome oxidase subunit 1 (12S, 16S, COX1). For primers used, see Table S1.

(4) For an additional phylogenomic verification of lineage relationships and resolution of deep nodes in the tree, we applied the FrogCap sequence capture strategy to sequence 12,951 nuclear-encoded markers from a set of selected samples, using methods described below.

Sequencing and sequence assembly

PCR products of datasets 1–3 were purified with Exonuclease I and Shrimp Alkaline Phosphatase digestion. The mitochondrial gene fragments were sequenced with the forward primer only, nuclear genes were sequenced on both strands and combined after careful inspection of reads to ensure correct identification of double peaks indicative of heterozygous nucleotide positions. Multiple heterozygous sites in two of the nuclear genes impeded accurate computational haplotype inference. We therefore selected a set of representative samples (7 samples for BDNF and 23 for RAG1) for which haplotypes were unreliably inferred, reamplified them using high fidelity *Pfu* polymerase (Promega), and cloned them using the TOPO TA Cloning Kit for Sequencing (Invitrogen). At least 8 clones per amplicon were sequenced for determination of haplotypes. Sequencing was performed on automated DNA sequencers at LGC Genomics (Berlin). Chromatograms were checked and edited with CodonCode Aligner 3.7.1 (Codon Code Corporation, Dedham, MA, USA) and newly determined sequences submitted to GenBank (accession numbers OQ001363–OQ001426, OQ023045–OQ023196, OQ059338–OQ060495). All sequences were quality-checked and trimmed using CodonCode Aligner, and then aligned in MEGA7 (Kumar et al. 2016) with the Muscle alignment option. A full table with all sequences, accession numbers, and associated metadata is available as Supplementary Material 1 and from the Zenodo repository (https://doi.org/10.5281/zenodo.7474341).

The sequence capture probe set used for assembling dataset 4 of this study is the FrogCap Ranoidea v2 probe set (Hutter et al. 2022; available at: https://github.com/chutter/FrogCap-Sequence-Capture). Probe design is described in detail by Hutter et al. (2022). We here summarize probe design and wet lab methods reproducing the methods descriptions from a previous paper (Rasolonjatovo et al. 2020): Probes were synthesized as biotinylated RNA oligos in a myBaits kit (Arbor Biosciences, formerly MYcroarray Ann Arbor, MI) by matching 25 publicly available anuran transcriptomes to the *Nanorana parkeri* and *Xenopus tropicalis* genomes using the program BLAT (Kent 2002). Sequences were matched to available coding region annotations from the *Nanorana* genome using BLAT (Sun et al. 2015). Markers from all matching species were then aligned using MAFFT (Katoh and Standley 2013) and subsequently separated into 120 bp-long bait sequences with 2× tiling (50% overlap among baits) using the myBaits-2 kit (40,040 baits) with 120mer sized baits. These loci also included adjacent intronic regions, ultra-conserved elements (UCEs) and commonly used Sanger-based legacy loci.

Genomic DNA was extracted from selected tissue samples using a PROMEGA Maxwell bead extraction robot, quantified, and used for library preparation by Arbor Biosciences library preparation service (Ann Arbor, Michigan, USA) using Illumina Truseq-style sticky-end library preparation. Following enrichment using the MYbaits v. 3.1 protocol, library pools were amplified for 10 cycles using universal primers and sequenced on an Illumina HiSeq X Ten with 150 bp paired-end reads. Raw reads can be found on the NCBI SRA: BioProject: PRJNA924698; BioSamples: SAMN32767203–SAMN32767214). The bioinformatics pipeline used for filtering adapter contamination, assembling markers, and exporting alignments has been described previously (Hutter et al. 2022). The pipeline is scripted in R statistical software (R Development Core Team, 2018) using the BIOCONDUCTOR suite of packages (Ramos et al. 2017) as well as FASTP (Chen et al. 2018). It involves merging paired-end reads using BBMerge (Bushnell et al. 2017) removing duplicates using "dedupe", and de novo assembly using SPADES v.3.12 (Bankevich et al. 2012) with BAYESHAMMER (Nikolenko et al. 2013) error correction. The final set of matching contigs was MAFFT-aligned, filtered, and trimmed, and markers retained when the number of samples in each alignment was greater than 75%. Alignments are available on the Zenodo repository (https://doi.org/10.5281/zenodo.7474341).

Analysis of molecular data

The four molecular datasets were separately analyzed as follows:

(1) To obtain a first understanding of mitochondrial differentiation among all available samples of *G. liber*, the cytochrome *b* alignment (dataset 1) was analyzed with a simple (K2P) substitution model to avoid overparametrization for shallow branches in a Maximum Likelihood analysis in MEGA 7 with NNI branch swapping, and 100 nonparametric bootstrap replicates to assess node support. Uncorrected pairwise distances between sequences for COB and 16S were calculated using the program TaxI2, implemented in iTaxoTools (Vences et al. 2021). We also used ASAP (Puillandre et al. 2020) to objectively delimit mitochondrial lineages within the *G. liber* complex for downstream taxonomic analysis, based on COB.

(2) The nuclear-encoded genes (RAG1, BDNF, TYR, POMC) were analyzed separately from the mitochondrial genes, and separately from 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 this gene. Haplotypes were estimated with the PHASE algorithm version 2.1.1 (Stephens et al. 2001). For BDNF and RAG1, cloned haplotypes were entered as "known" into the PHASE program after preparing input files in

SeqPhase (Flot, 2010). We specified 1000 burn-in steps and 1000 iterations and ran each nuclear gene alignment four times to check for consistency. The phased sequences were then used to reconstruct Maximum Likelihood trees with the Jukes-Cantor substitution model in MEGA 7, and these then 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).

(3) The multigene dataset of a representative number of samples was first analyzed with PartitionFinder v. 2.1.1 (Lanfear et al. 2017) to determine the best scoring partitioning scheme and appropriate substitution models, and was then submitted to BI searches in MrBayes (Ronquist et al. 2012). Table S2 gives the substitution models and partitions applied for the multigene phylogenetic reconstruction. We ran the analysis for 20 million generations, and chains were sampled every 1000 generations. A relative burn-in of 25% of the samples was conservatively discarded after assessing MCMC convergence.

(4) Phylogenomic (FrogCap sequence capture) data were analyzed under maximum-likelihood in IQ-Tree v. 1.6.7 (Nguyen et al. 2015), based on a by-marker partitioned data matrix and models of molecular evolution identified via ModelFinder (Kalyaanamoorthy et al. 2017), and with 1000 ultrafast bootstrap replicates. To perform species tree estimation to address potential incomplete lineage sorting (ILS), we use the software ASTRAL-III (Zhang et al. 2018), which conducts a summary-coalescent species tree analysis that is statistically consistent under the multi-species coalescent model. As input for ASTRAL-III, we performed maximum likelihood (ML) analyses on each alignment using IQ-Tree. To improve accuracy, we collapsed branches that were below 10% bootstrap support, as recommended by the authors. Exploratory analyses were also performed for partial data matrices consisting of UCEs, exons, and introns, and different levels of missing data (50% and 75%) which resulted in topologies (not shown) compatible with those of the analysis of the full dataset 4.

Because some of the species distinguished herein according to available data cannot be reliably diagnosed based on morphology, we provide a molecular diagnosis to satisfy the requirements of the Code for diagnostic traits that purport to distinguish each new species from all previously described species. For molecular diagnosis we used the tool DNAdiagnoser implemented in iTaxoTools (Vences et al. 2021) to identify and tabulate pairwise diagnostic differences between species for the cytochrome *b* fragment. For this purpose, we used a trimmed cytochrome *b* alignment of 516 bp for 306 *Guibemantis* individuals, and provide the position of the diagnostic sites relative to the full cytochrome *b* sequences of the mantellid frog *Mantella baroni* (accession number NC_039758).

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 used this to assign species status to lineages, along with concordance between genetic, bioacoustic, and morphological evidence (Padial et al. 2010).

Results

Genetic variation and delimitation of lineages

The cytochrome *b* alignment (dataset 1) consisted of 338 sequences of the *G. liber* complex, plus 56 sequences of other *Guibemantis* species and hierarchical outgroups (*Gephyromantis*, *Spinomantis* and *Boophis*), for an alignment length of 545 nucleotides. A ML tree based on this dataset is shown in Fig. 1. Our delimitation of lineages applied for downstream taxonomic analysis relied on an ASAP analysis of this dataset. Based on the calculation of pairwise distances and the choice of the lowest ASAP score (score = 1.0) that indicated the best partition available, nine ingroup mitochondrial lineages were delimited. For further analysis, illustration and discussion, we named these lineages based mainly on their geographic distribution: northern lineage (NOR; Montagne d'Ambre), north-central lineage (NCENTR; Ambodiriana, Bemanevika, Makira, Tsaratanana), north-central eastern 1 (NCE1; An'Ala, Andasibe, Besariaka, Mandraka, Mangoro River, Vatomandry), north-central eastern lineage 2 (NCE2; Ambodisakoa, Ambohitantely, Andasibe, Andranomapanga, Anjozorobe, Mahasoa, Vatomandry), north-central coastal lineage (NCC; Sahafina), south-central eastern lineage (SCE; Maharira, Mananjary, Ranomafana, Samalaotra/Ifanandiana, Vohiparara) and the south-eastern lineage (SOE; Manombo, Vevembe); as well as the two particularly distinct lineages mostly found in the North East, NE1 (Ambodivoangy, Bemanevika, Makira, Marojejy Camp Simpona, Tsaratanana) and NE2 (Andrakata/Andapa and Marojejy Camp Mantella). See Fig. 2 for the geographical distribution of the mitochondrial lineages and of our sampling sites.

The minimum and maximum pairwise uncorrected cytochrome *b* distances between the nine main lineages of the *Guibemantis liber* complex (Table S3) revealed a very high genetic distance of the northern lineages NOR and NCENTR, to the other, partly sympatric north-eastern lineages NE1 (13.8–15.5% and 13.4–15.5%) and NE2 (13.9–14.7% and 13.4–14.2%) (co-occurrence demonstrated for NCENTR and NE1 at Makira, Bemanevika and Tsaratanana; see Supplementary Material 1 and 2). These values

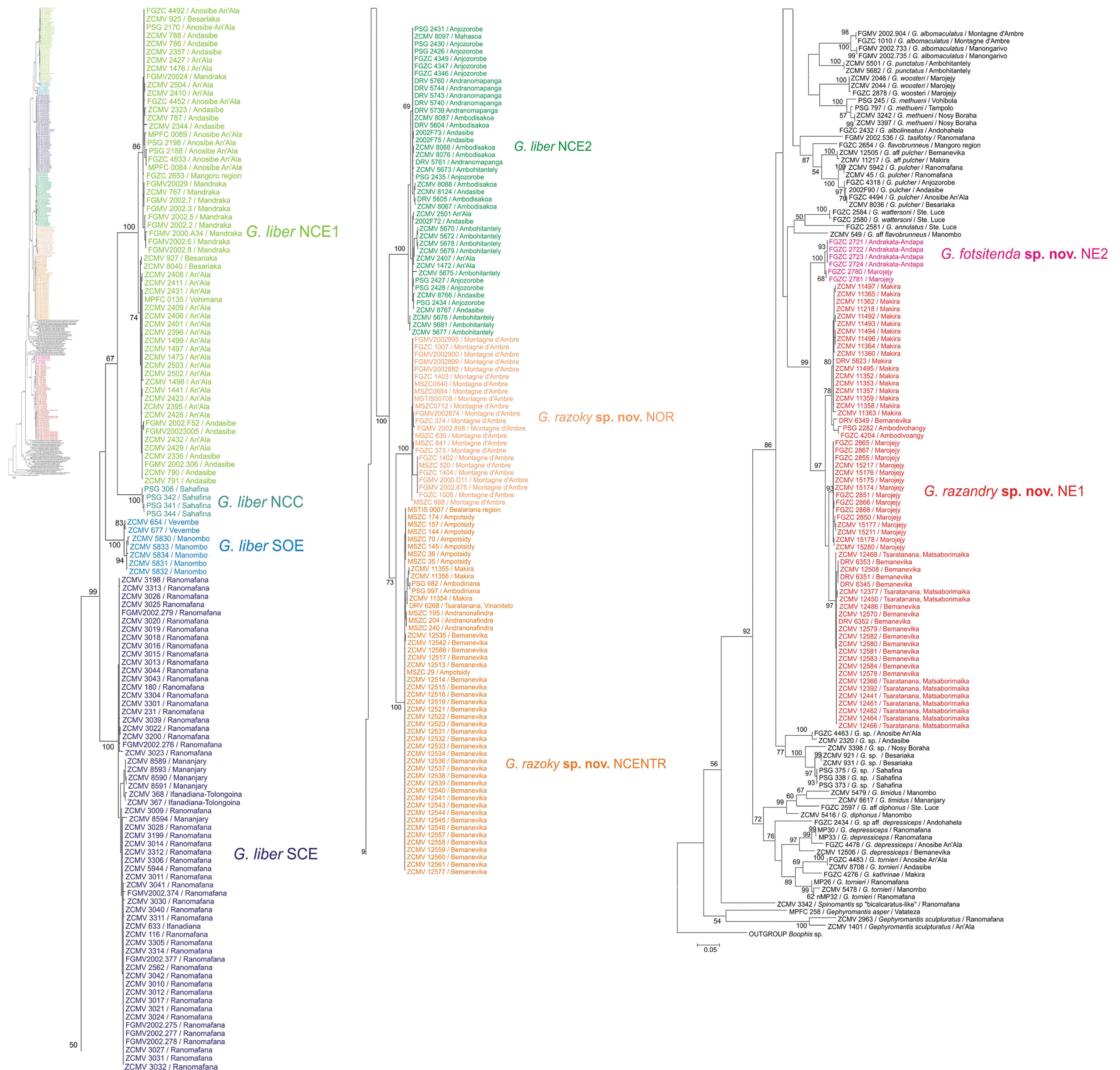

Figure 1. Maximum likelihood phylogeny based on DNA sequences of the mitochondrial cytochrome *b* alignment (dataset 1; alignment length 545 nucleotides) for 338 sequences of the *G. liber* complex, plus 56 sequences of other *Guibemantis* species and outgroups. Numbers at nodes are support values from a bootstrap analysis (100 replicates; values <50% and values of most of the shallow nodes not shown). The tree was rooted with a set of hierarchical outgroups of the mantellid genera *Boophis*, *Gephyromantis* and *Spinomantis* (removed from the figure for better graphical representation).

are higher than typical intra-species distances which in cytochrome *b* are typically <10% in tropical anurans (Rodríguez et al. 2015). The two north-eastern lineages NE1 and NE2 show a somewhat lower genetic divergence between each other (7.0–8.9% in cytochrome *b*), and the genetic distance between the northern lineages NOR and NCENTR is even lower (5.3–7.2%).

Substantial genetic divergence was also observed between the northern and central/southern lineages, e.g., when comparing NOR and NCENTR with NCE1 (11.2–12.8% and 10.9–12.5%) and NCE2 (8.1–9.7% and 7.6–9.3%). Even between the two southern lineages SCE and SOE a genetic distance of 7.8–9.7% was found. The lineage NCC, only identified from Sahafina, differs from the geographically adjacent NCE1 by 8.7–10.7%.

Genetic distances in a fragment of the 16S rRNA gene that has often been used for DNA barcoding of Malagasy frogs (e.g., Vieites et al. 2009) and for which numerous comparative data are available, revealed divergences in some cases substantially exceeding the 3% threshold defined by Vieites et al. (2009) to define candidate species (Table S4): 7.1–7.8% / 6.1–7.1%, and 7.8–7.9% / 6.5–7.1% comparing NOR and NCENTR to NE1 and NE2; 2.8–3.9% between NE1 and NE2; 3.9–4.7% / 2.4–3.3%, and 3.5–5.5% / 2.9–5.0% comparing NCE1 and NCE2 with NOR and NCENTR; and 2.9–3.9% / 4.6–5.4%, and 3.8–6.2% / 4.3–6.4% comparing SCE and SOE with NCE1 and NCE2. NE1 and NE2 show a divergence of 5.3–10.1% / 6.2–9.8% compared to all other non-sympatric lineages (NCE1, NCE2, NCC, SCE, SOE).

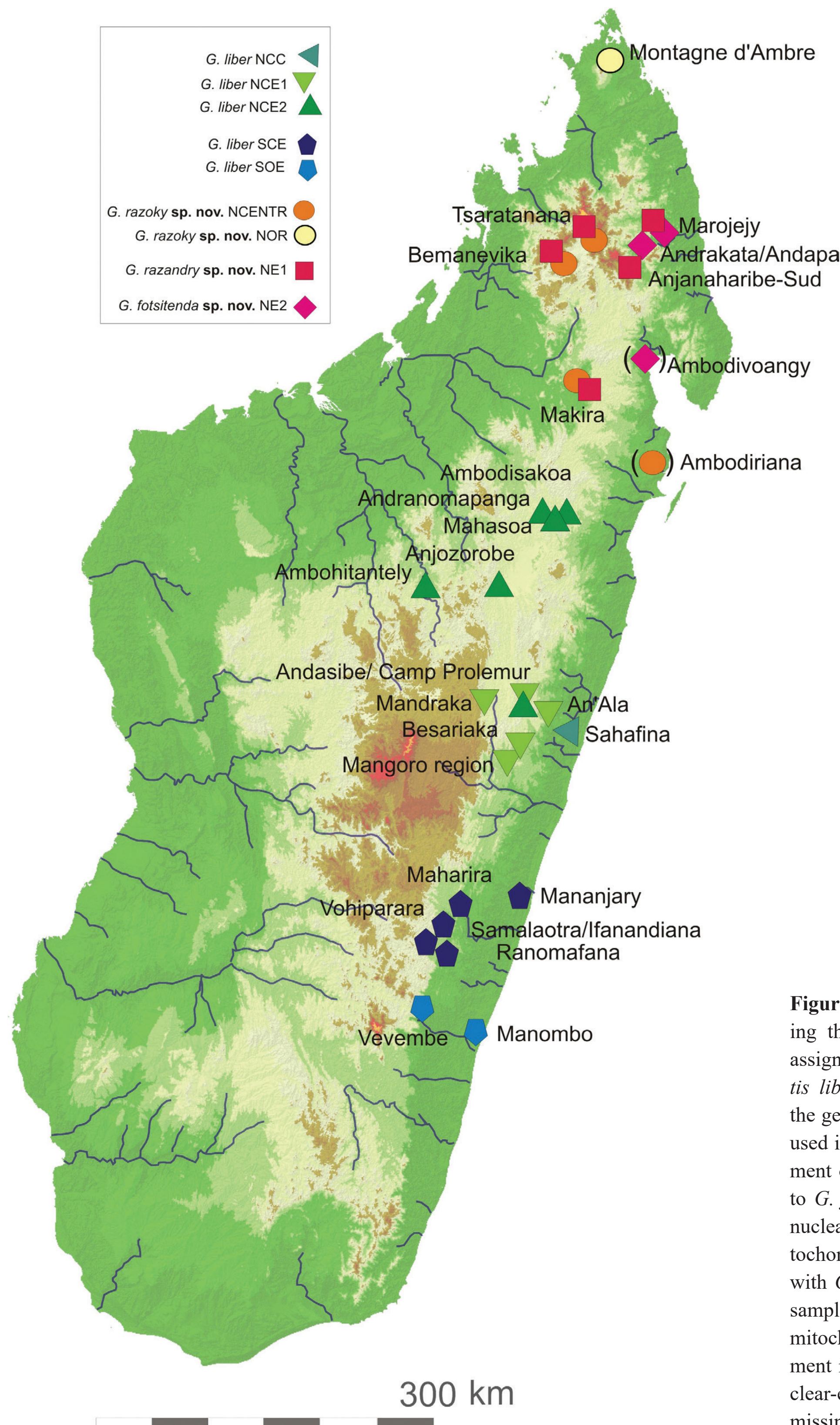

Figure 2. Map of Madagascar showing the confirmed localities of the assigned lineages of the *Guibemantis liber* complex (color scheme of the genetic lineages refers to the one used in Fig. 1). Note that the assignment of the Ambodivoangy samples to *G. fotsitenda* relies on data from nuclear-encoded genes, whereas mitochondrial DNA place these samples with *G. razandry*. The Ambodiriana samples are assigned to *G. razoky* by mitochondrial DNA but this assignment is in need of confirmation (nuclear-encoded DNA sequences are missing for these samples).

The alignments of the four nuclear-encoded genes, not counting the outgroup sequences, consisted of 257 sequences of the *G. liber* complex (514 nucleotides) for RAG1, 244 sequences (577 nucleotides) for BDNF, 245 sequences (405 nucleotides) for POMC, and 153 sequences (548 nucleotides) for TYR (sequence numbers doubling after phasing, respectively). Inspection of the haplotype networks resulting from these four nuclear-encoded genes (Figs 3–6) revealed haplotype sharing between the northern lineages NOR and NCENTR for RAG1, BDNF and POMC. For NCE1 and NCE2 extensive haplotype sharing was present in all four nuclear-encoded genes examined, and no differentiation in nuclear genes was obvious at An'Ala where NCE1 and NCE2 mitochondrial haplotypes

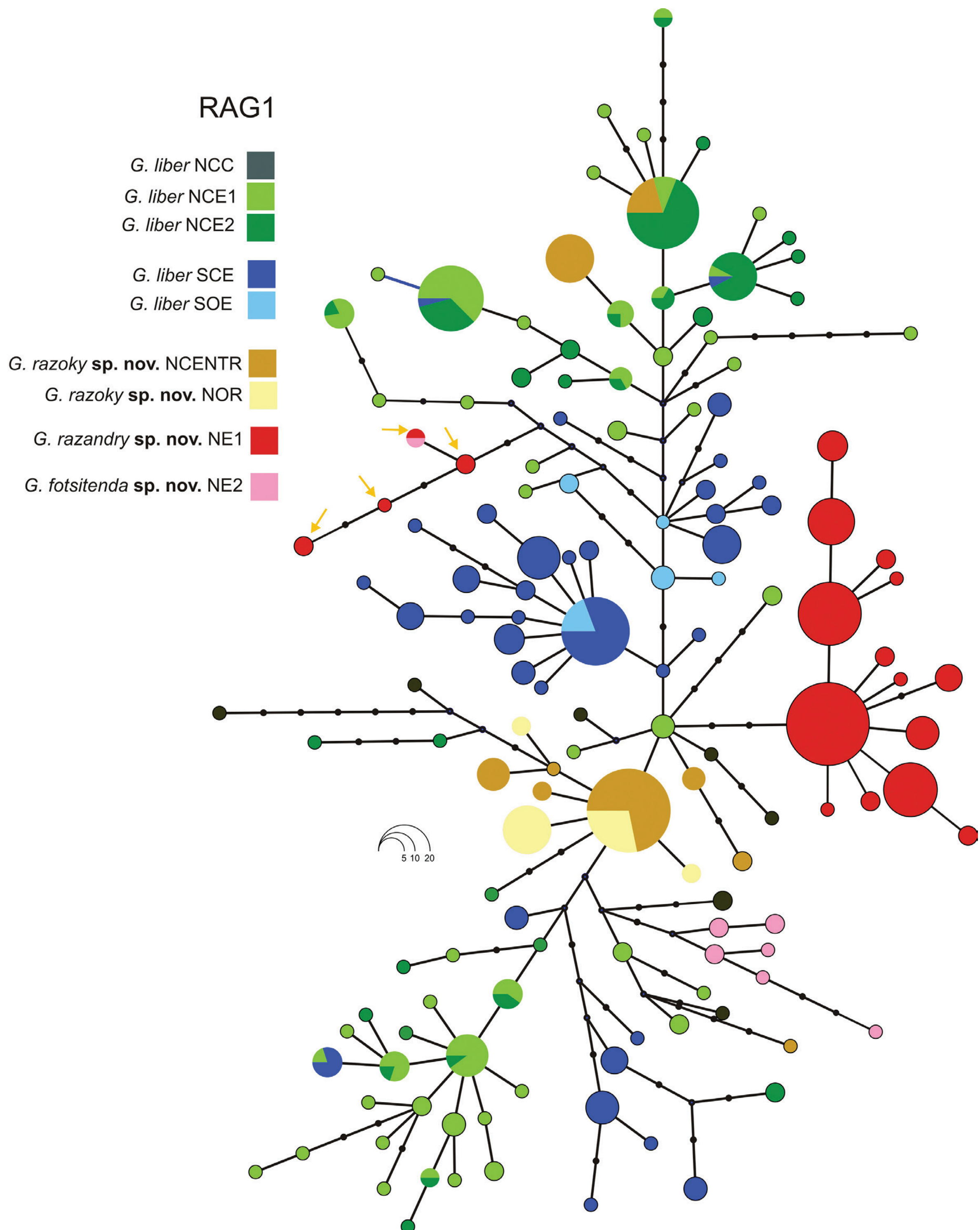

Figure 3. Haplotype network reconstructed from 257 phased DNA sequences of the *G. liber* complex (514 nucleotides) of the RAG1 gene. Sequences were colored according to the assignment of the respective individuals to mitochondrial lineages. Orange arrows indicate samples from Ambodivoangy which are colored red as they belong to the mitochondrial lineage NE1, but cluster with samples of lineage NE2 in the nuclear-encoded genes (see Fig. S2).

occurred in syntopy (Fig. S1). The north-eastern lineages NE1 and NE2 were separated from the NCENTR lineage, with which NE1 occurs in sympatry in some populations, for all genes except for POMC gene where some haplotype sharing was detected. For BDNF, NE1/NE2 shared haplotypes only with the NCE1 clade (also in RAG1). In most networks, NE1 and NE2 appear to share haplotypes with each other; however, closer examination (Fig. S2) revealed that this was only true when considering samples from Ambodivoangy as NE1 based on mitochondrial DNA; these samples clustered with NE2 samples based on all informative nuclear markers, suggesting they probably belong to the NE2 evolutionary lineage but possess an introgressed mitochondrial genome from NE1.

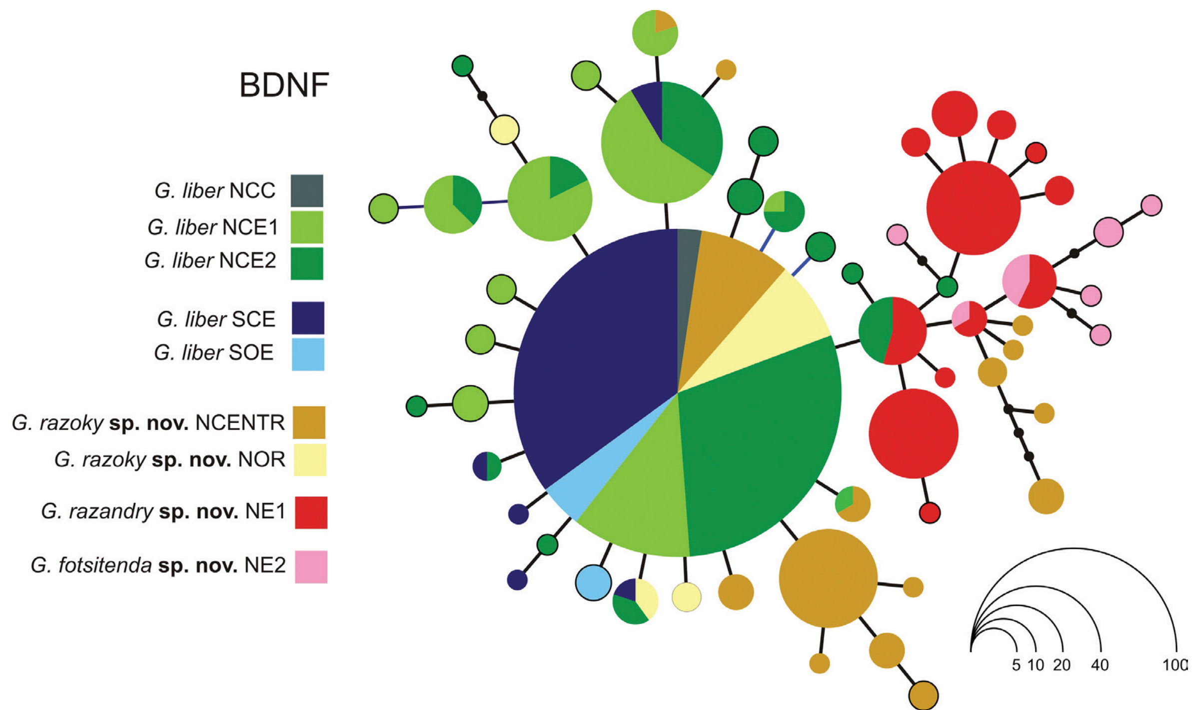

Figure 4. Haplotype network reconstructed from 244 phased DNA sequences of the *G. liber* complex (577 nucleotides) of the BDNF gene. Sequences were colored according to the assignment of the respective individuals to mitochondrial lineages.

Phylogenetic relationships

The ML tree inferred from the cytochrome *b* dataset (Fig. 1) illustrates the genetic divergence between these nine distinct mitochondrial lineages and provides initial hints on their evolutionary relationships, although most of the deep nodes in the tree are not supported by bootstrap analysis. For in-depth phylogenetic analysis we therefore carried out combined mtDNA/nucDNA and FrogCap analyses; see below. In the cytochrome *b* tree, the genetically most divergent clades comprising the northern lineages NE1 and NE2 from Ambodivoangy, Andrakata, Andapa, Bemanevika, Makira, Marojejy and Tsaratanana were clearly separated and placed sister to each other with 99% bootstrap support. Both together formed the sister group to *G. annulatus*, *G.* aff. *flavobrunneus* and *G. wattersoni*, and this entire group together were in turn sister to *G. albolineatus*, *G. albomaculatus*, *G. bicalcaratus*, *G. flavobrunneus*, *G. pulcher*, *G.* aff. *pulcher*, *G. punctatus*, *G. tasifotsy*, *G. woosteri*, and all the other representatives of the *Guibemantis liber* complex, although this pattern – and thus the paraphyly of the *G. liber* complex – remained without bootstrap support from this analysis. Although specimens assigned to the lineages NCENTR occur in sympatry – and even syntopy – with the north-eastern lineage NE1 at least in Bemanevika, Makira, and Tsaratanana, they clustered with the other northern lineage NOR from Montagne d'Ambre with 73% bootstrap support (100% support for NOR). The NCENTR/NOR clade was sister to NCE2, distributed south of the NCENTR range in Ambodisakoa, Ambohitantely, Andasibe, Andranomapanga, Anjozorobe, Mahasoa and Vatomandry, with 100% bootstrap support (100% support also for NCE2). Individuals of NCE2 were found in sympatry with representatives of NCE1 in central eastern Madagascar (Andasibe and An'Ala). NCE2, NOR and NCENTR were placed as a sister group to NCE1, NCC, SOE and SCE (each of these lineages supported with 100% bootstrap support), with 50% bootstrap support. NCE1 was sister to NCC from Sahafina, with 67% bootstrap support.

Partitioned Bayesian phylogenetic inference of the combined mitochondrial and nuclear-encoded gene fragments (COB, COX1, 12S, 16S, RAG1, RAG2, BDNF, POMC, TYR) for representative samples (Fig. 7) confirmed the existence of strong divergence within the *G. liber* complex, and most of the relationships as already suggested by the initial tree based on cytochrome *b* only (Fig. 1). All previously defined lineages were supported in the combined tree with posterior probabilities (PP) of 1.0. The NE1/NE2 clade was placed separate from the NOR/NCENTR clade, and instead formed the sister group to those and all the other lineages of the *G. liber* complex, plus *G. albolineatus*, *G. annulatus*, *G. pulcher*, *G.* aff. *pulcher*, *G. tasifotsy*, *G. wattersoni*, with a low PP of 0.89. The NOR/NCENTR clade plus NCE2 was the sister group (PP = 1.0) to a second clade containing NCE1+NCC (which formed a clade with PP = 1.0), and the southern SOE+SCE lineages (which formed a clade with PP = 1.0).

The FrogCap procedure carried out on 11 representative samples of *G. liber*, representing all main lineages except for SCE and NCC, and one outgroup (*G. depressiceps*) yielded a total of 12,951 nuclear-encoded markers of an average length of 849 nucleotides across all samples and markers. Of these, between 165–1561 were missing for the various samples, with the highest number of 12% missing data corresponding to the outgroup. A partitioned

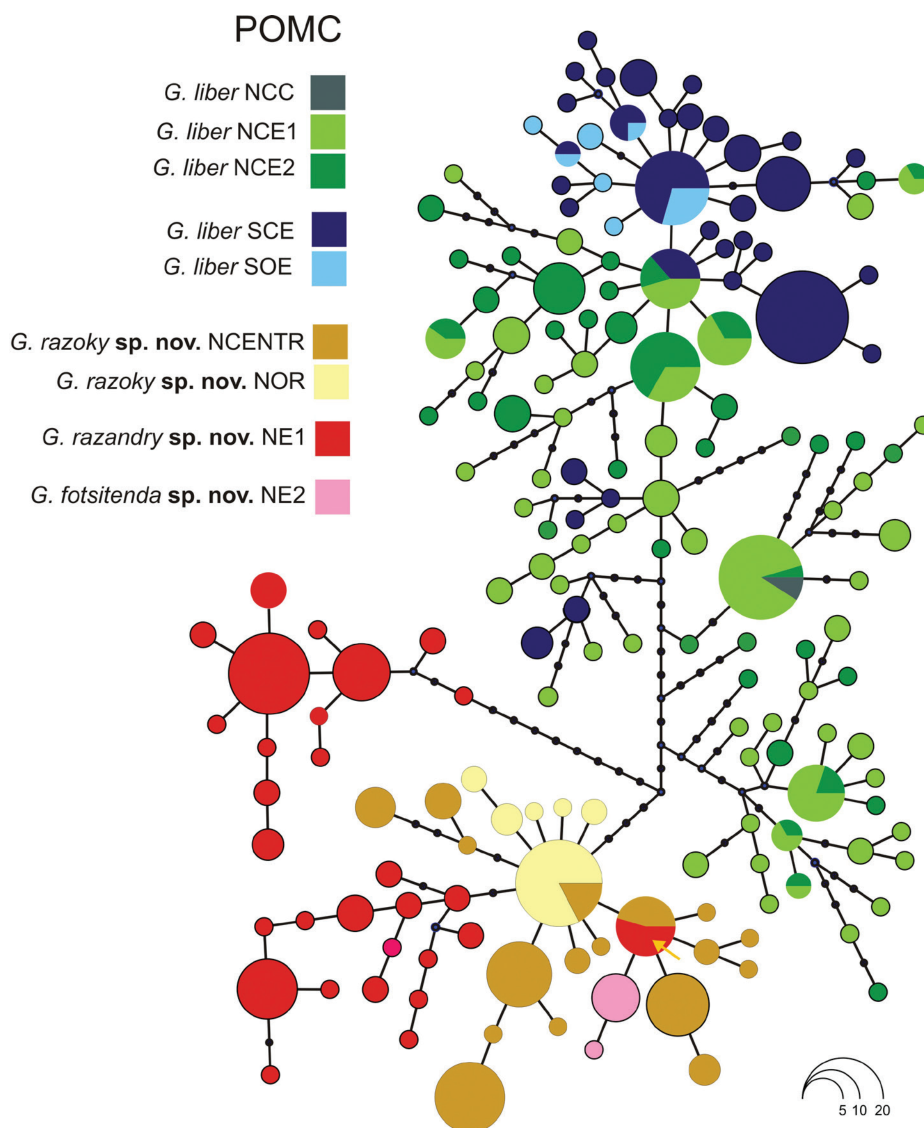

Figure 5. Haplotype network reconstructed from 245 phased DNA sequences of the *G. liber* complex (405 nucleotides) of the POMC gene. Sequences were colored according to the assignment of the respective individuals to mitochondrial lineages. The orange arrow indicates samples from Ambodivoangy which are colored red as they belong to the mitochondrial lineage NE1, but are placed closer samples of lineage NE2 in the nuclear-encoded genes (see Fig. S2).

maximum likelihood analysis of this dataset inferred a fully resolved tree (Fig. 8) with maximum bootstrap support of 100% at all nodes. The two northeastern lineages were recovered as sister groups separated by comparatively long branches, and together formed the sister group of all other lineages. The northern/north-central lineages together were sister to the NCE1, NCE2 and SOE lineages. A species tree analysis with ASTRAL-III (Fig. S3) recovered the same relationships with maximum support except for the placement of *G. liber* SOE, which was placed sister to the clade of NOR/NCENTR plus the remaining *G. liber* lineages.

Morphological variation

All specimens of the *G. liber* complex included in our morphological analysis were highly variable in color pattern and similar to each other in morphometry (Figs 9–12, Table 1). Clear and constant differences in coloration from the available pictures in life could not be detected. Only a tendency for differentiation in coloration and pattern was recognizable for the following lineages: yellow ventrolateral blotches posteriorly (in the inguinal region) are typical for the northern lineages NCENTR and NOR and present in most other lineages but are largely absent in specimens of the north-eastern lineages NE1 and NE2. Specimens from Sahafina (lineage NCC, not sampled for nuclear genes) tend to show distinct and broader black vertical arrow-like stripes on the iris that point to the pupils (Fig. 10).

Some differences among lineages seem to exist in body size: When comparing male specimens (for female specimens see Table 1), snout–vent length (SVL) differed particularly when comparing the northern lineages. Individuals assigned to NOR (SVL 29.3–33.9 mm) and NCENTR (26.6–31.5 mm, with only a single male smaller than 28.0 mm) tend to be larger than those of the (partly syntopic) lineages NE1 (21.8–27.4 mm) and NE2 (25.1–26.0 mm).

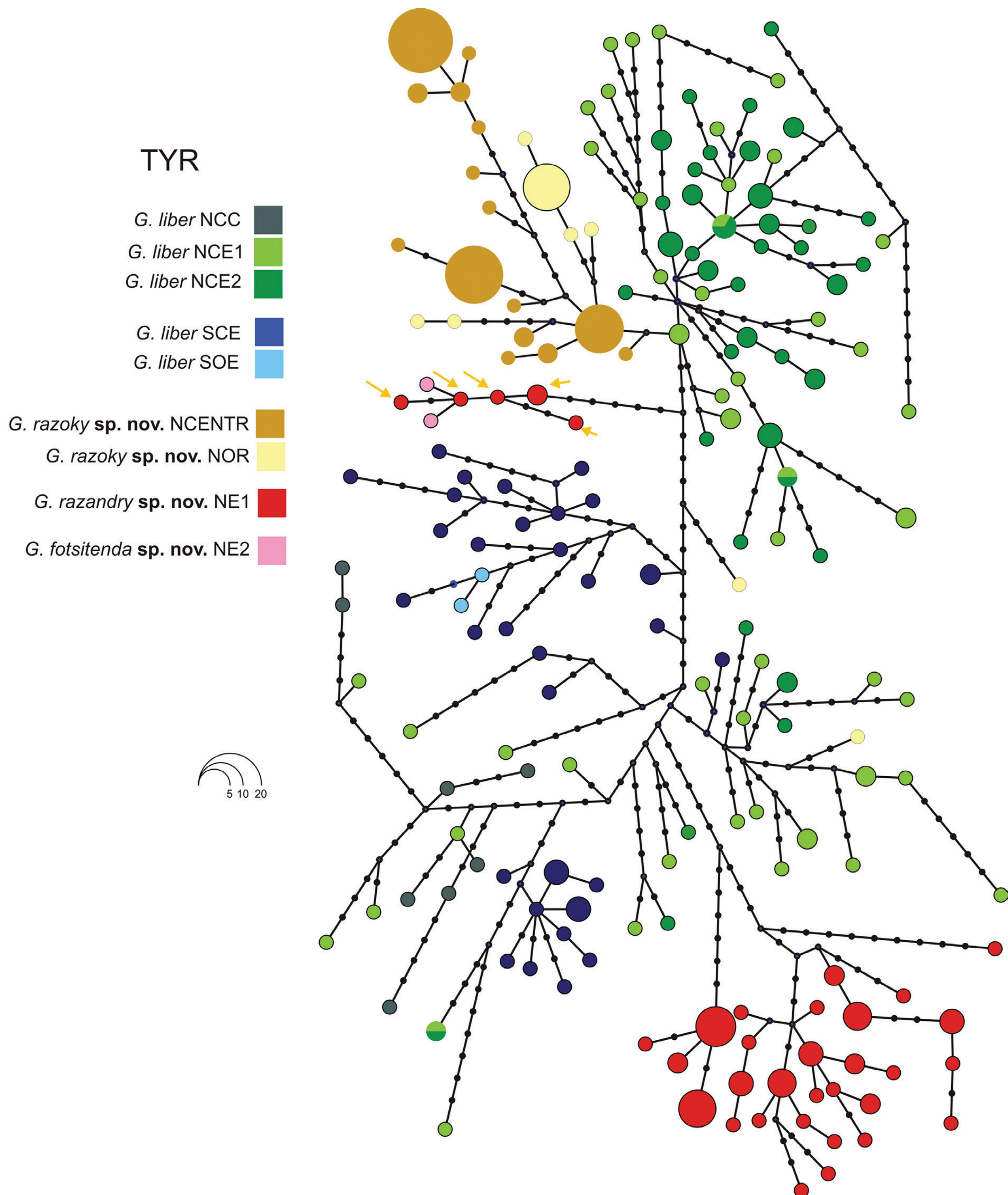

Figure 6. Haplotype network reconstructed from 153 phased DNA sequences of the *G. liber* complex (548 nucleotides) of the tyrosinase gene (TYR). Sequences were colored according to the assignment of the respective individuals to mitochondrial lineages. Orange arrows indicate samples from Ambodivoangy which are colored red as they belong to the mitochondrial lineage NE1, but cluster with samples of lineage NE2 in the nuclear-encoded genes (see Fig. S2).

Additionally, some specimens of the lineages NCE1 and NCE2 also show a relatively large SVL. The NE1 specimens from Bemanevika (21.8–24.3 mm) are the smallest ones examined, although some of the individuals could not be reliably sexed and thus may be immature. While NE1 and NE2 in general tend to be smaller than all other lineages of the *G. liber* complex, they do show an overlap particularly with specimens of the southern lineages SCE (24.2–27.1 mm) and SOE (25.7–27.8 mm).

Bioacoustic variation

Vocalizations in the *Guibemantis liber* species complex exhibit rather variable patterns. As a general observation, males of probably all identified lineages in this complex can emit at least two different note types: simple, short click-notes, and longer notes, often pulsed, and usually emitted in series. Both note types can be combined in different and complex ways. Moreover, alteration of notes

Figure 7. Majority-rule consensus tree from a partitioned Bayesian phylogenetic inference based on the combined mitochondrial and nuclear-encoded gene fragments (COB, COX1, 12S, 16S, RAG1, RAG2, BDNF, POMC, TYR) for representative samples of the genetic lineages of the *G. liber* complex and all other nominal species of *Guibemantis*. Values at nodes are Bayesian posterior probabilities (not shown if <0.89 and for some of the shallowest nodes. The tree was rooted with a species of the mantellid genus *Mantella* (removed from the figure for better graphical representation). See Discussion for an evaluation of the apparent paraphyly of the *G. liber* complex suggested by this tree.

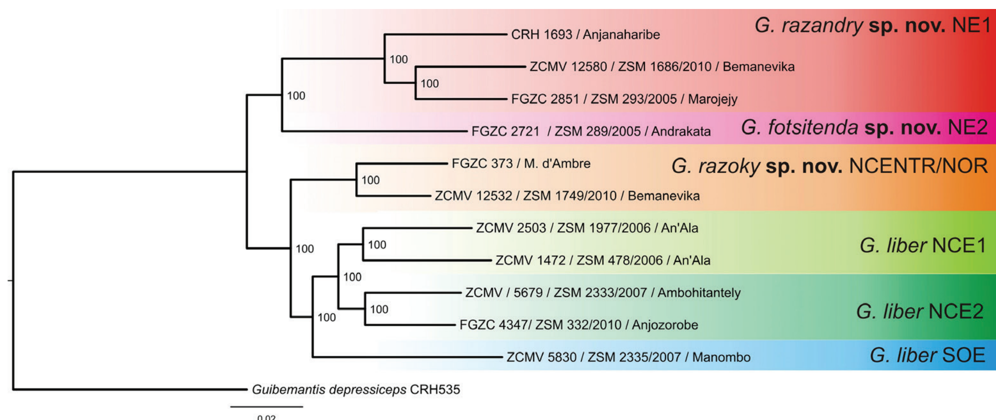

Figure 8. Maximum likelihood tree based on a partitioned analysis of 12,951 nuclear-encoded markers obtained via the FrogCap strategy for representative samples of the main lineages of the *G. liber* complex, calculated with IQ-Tree. Numbers at nodes are bootstrap values in percent.

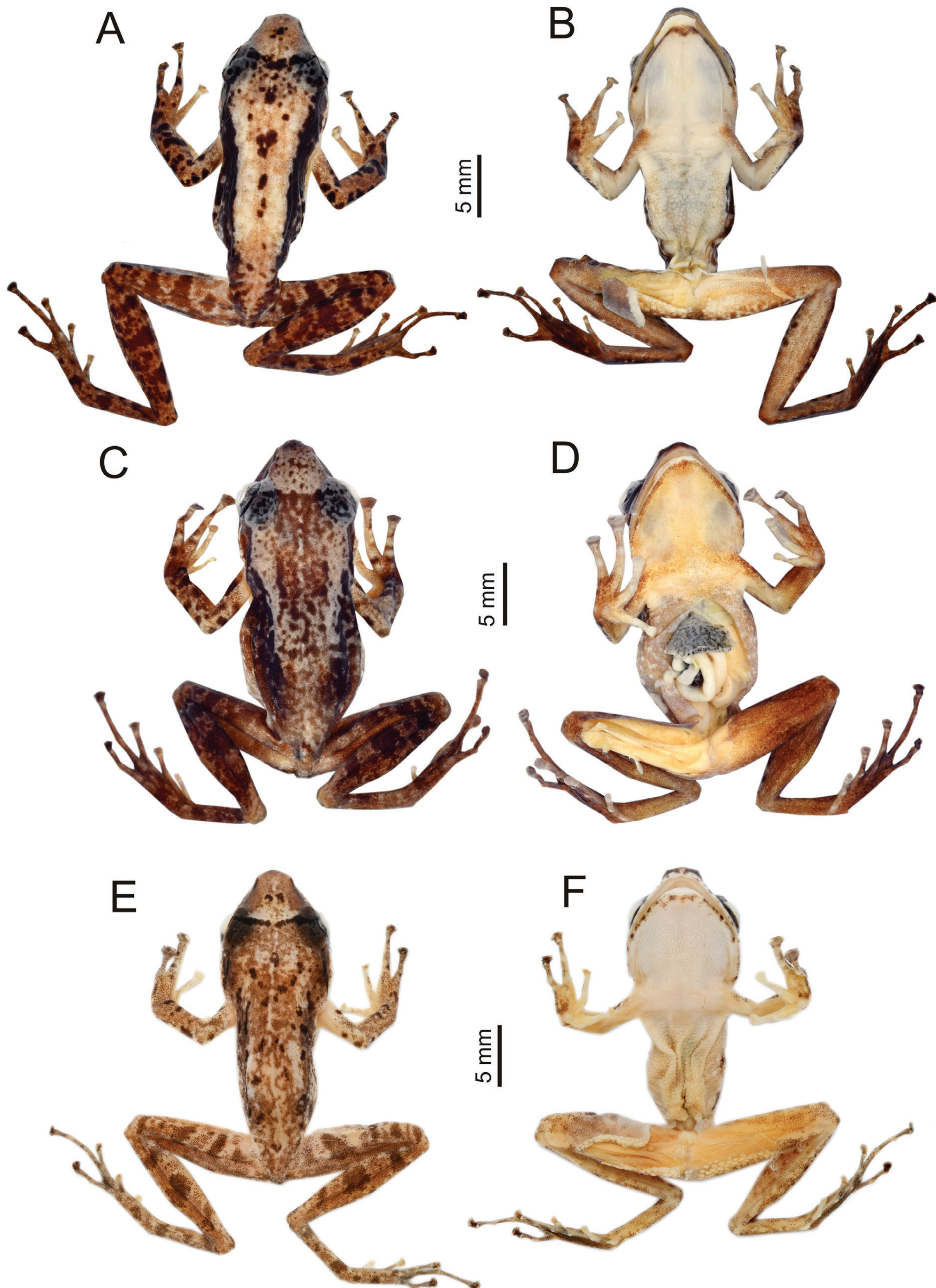

Figure 9. Preserved name-bearing holotype specimens of *Guibemantis razandry* **sp. nov.** (ZSM 293/2005) (**A**, **B**), *Guibemantis razoky* **sp. nov**. (ZSM 1746/2010) (**C**, **D**), and *Guibemantis fotsitenda* **sp. nov.** (ZSM 292/2005) (**E**, **F**) in dorsal and ventral view.

may result in intermediate note structures and continuous transition from one note type to the other seems possible. These phenomena make analysis of calls difficult, even more so as these frogs usually call within larger choruses composed of multiple individuals (Fig. 13) and therefore allocation of a certain sound on a recording to a particular individual is challenging.

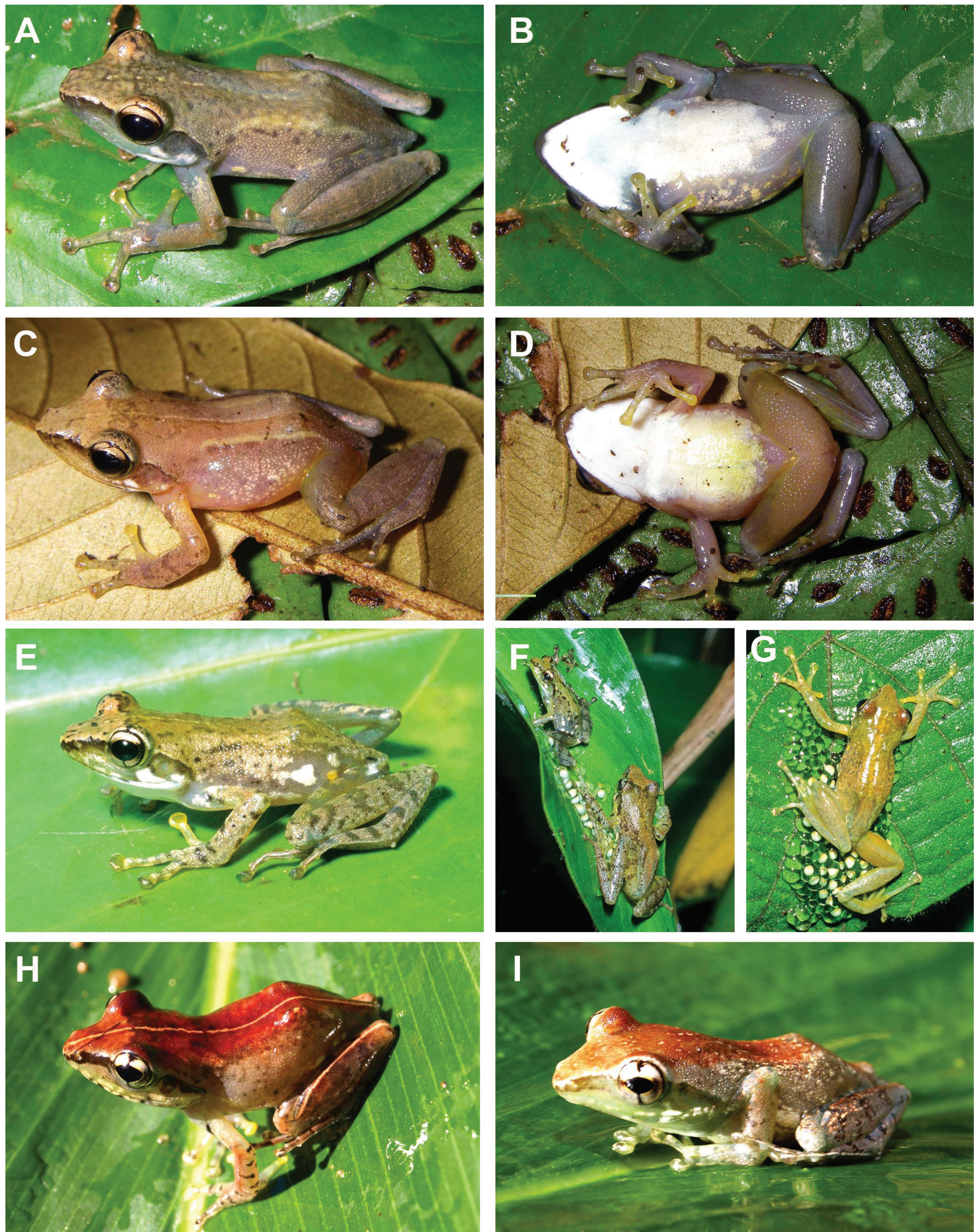

Figure 10. Specimens of *Guibemantis liber* in life, in dorsal and ventral views. **A**–**D** Male specimens from Mahasoa assigned to lineage NCE2, photographed in 2008. **E** Male specimen and **F** male and female specimens (not collected) from Mandraka assigned to lineage NCE1, photographed in 2000. **G** Female specimen (not collected) from Ranomafana assigned to lineage SCE, photographed in 2004. **H**, **I** Male specimens (not collected) from Sahafina assigned to lineage NCC (note the blackish arrow-like stripes on the iris), photographed in 2010.

As observed in many other groups of frogs, we may assume that the different note types recognized in the *G. liber* complex have different functions. In our experience and given the social context in which recordings were obtained, it seems plausible that the simple click-notes, mostly emitted at irregular intervals and very often not

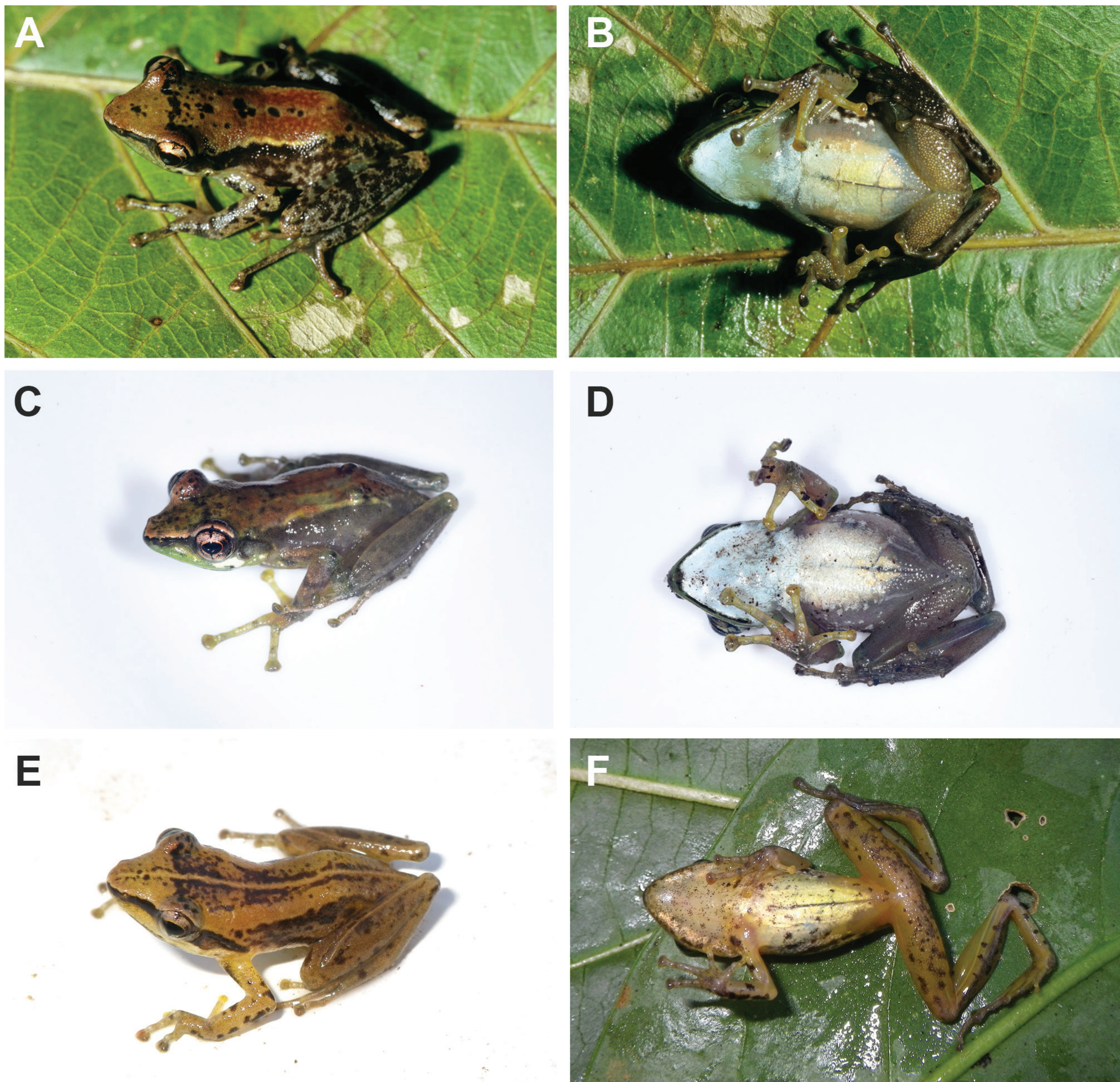

Figure 11. Specimens of *Guibemantis razandry* **sp. nov.** in life, in dorsal and ventral views. **A**, **B** Male holotype ZSM 293/2005 (field number FGZC 2851) from Marojejy. **C**, **D** Male specimen from Marojejy, photographed in 2016 (not collected). **E**, **F** Specimen from Bemanevika, probably a (maybe subadult) female, assigned to the species by color pattern only, photographed in 2010 (not collected).

following a particular pattern of repetition, have a territorial function, whereas the notes emitted in regular series likely have an advertisement function. In our analyses of calls and call comparisons we therefore focus on the notes emitted in series and tentatively classify them as advertisement calls. This action is supported by the minor differences among recorded simple click-notes within this species complex that are lesser than those expected to represent species-specific call differences (see Köhler et al. 2017). However, given the statements above and the sparse number of recordings available, our analysis of bioacoustic differentiation has to be taken with some reservation.

Among the lineages with calls analyzed, some rather clear differences in temporal structure and spectral character are evident, as summarized in the following. For detailed call descriptions, see the accounts of new species below, and Appendix 1 which also includes spectrograms and oscillograms (Figs 14–18).

Calls assignable to the lineage NCE1 from Mandraka and Andasibe contain a comparatively high number of pulses (11–22), with call energy distributed in a rather narrow frequency band only (prevalent bandwidth 1800–3500 Hz). Calls from An'Ala are very similar in character but are shorter and thus contain a lower number of pulses (7–12). These An'Ala calls are possibly assignable to NCE1 as well, as pulse rate within calls is rather similar among all three localities, ranging from approximately 140–170 pulses/second.

Calls from Ambodisakoa (near Mahasoa), assignable to lineage NCE2, are similar in overall character compared to those of NCE1, but differ by longer call duration

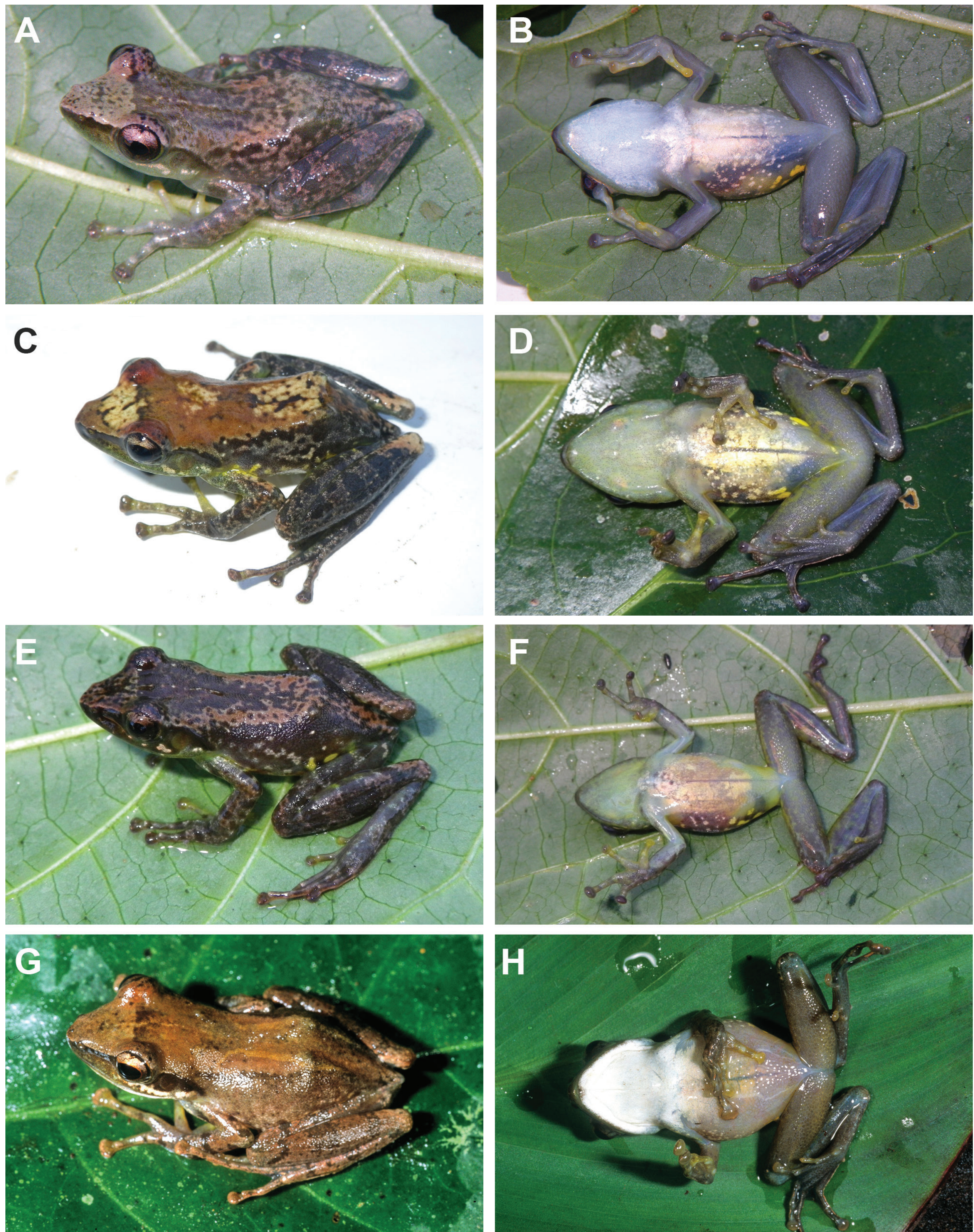

Figure 12. Specimens of *Guibemantis razoky* **sp. nov**. in life, in dorsal and ventral views. **A**, **B** Male holotype ZSM 1746/2010 (field number ZCMV 12515) from Bemanevika. **C**, **D** Female paratypes ZSM 1744/2010 (field number ZCMV 12513), and **E**, **F** ZSM 1745/2010 (field number ZCMV 12514) from Bemanevika. **G**, **H** Male paratype from Montagne d'Ambre (ZSM 878/2003) (shown in Lehtinen et al. 2012 as *G. liber*).

(135–195 vs. 49–131 ms), higher pulse rate within calls (255–285 vs. 40–170 pulses/second), and distinctly lower call repetition rate in call series (28–30 vs. 145–251 calls/minute).

Calls assigned to lineage SCE from the Ranomafana area exhibit the shortest call duration (21–35 ms) among all calls analyzed. Furthermore, call energy is distributed across a very limited frequency band only (prevalent

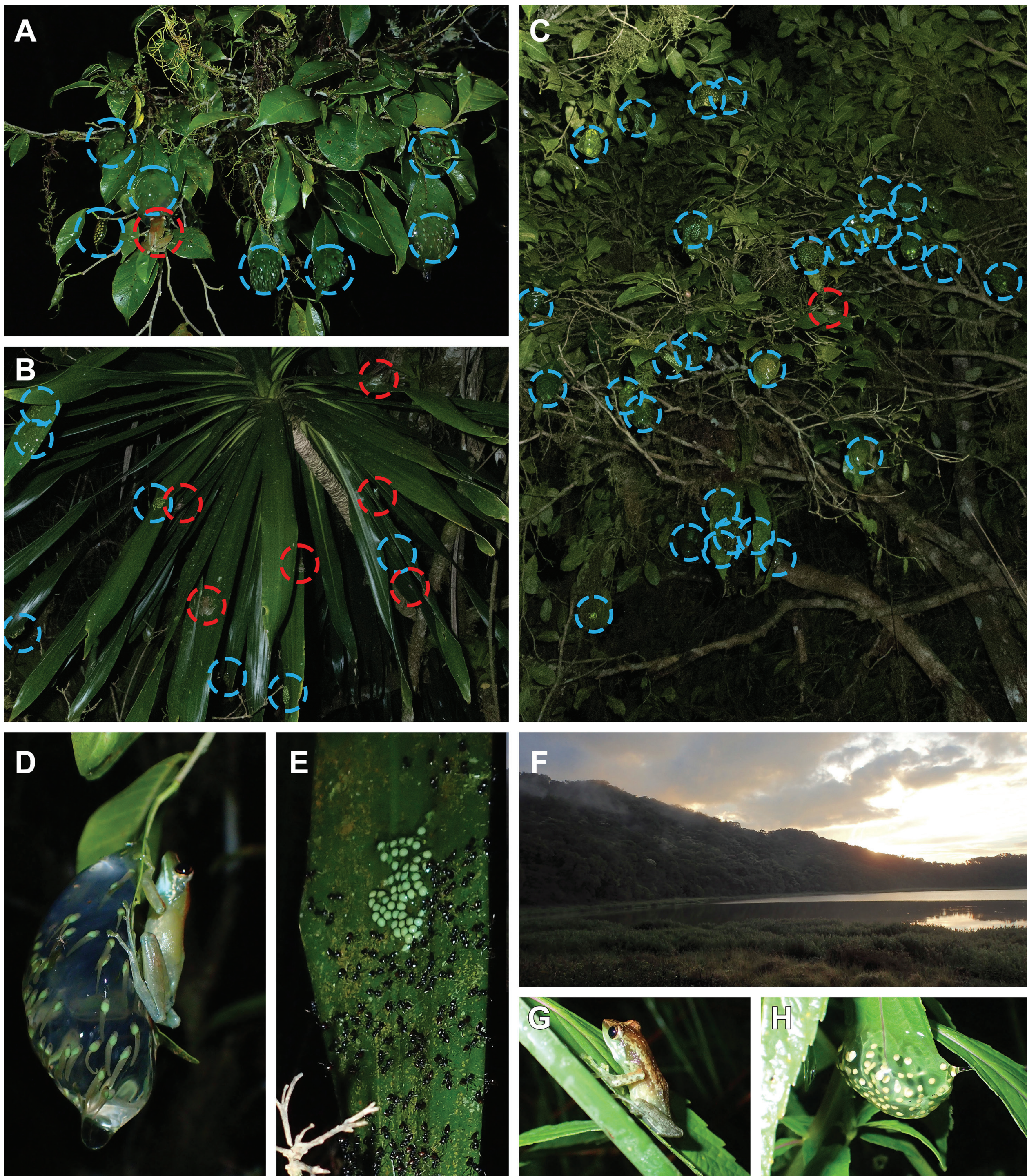

Figure 13. Breeding assemblages of *Guibemantis razoky* **sp. nov.** on Montagne d'Ambre. **A**–**E** Scenes from a single locality where dozens of specimens and hundreds of clutches were found, alongside individuals of *Blommersia wittei* and *Guibemantis albomaculatus*. Red circles are *G. razoky* **sp. nov.** individuals, blue circles are clutches. **E** Ants swarming eggs on a *Pandanus* frond. **F** Lac Maudit (~1320 m a.s.l.) at sunset. **G** *Guibemantis razoky* **sp. nov.**, and **H** a clutch of their eggs from the bank of Lac Maudit.

bandwidth 2700–3500 Hz). In quantitative parameters and partly also structure, these calls differ from *G. liber* calls further north (NCE lineages). However, only a limited number of recordings, partly of low quality, are thus far available from the southern lineages.

Calls from Montagne d'Ambre, assignable to lineage NOR, are distinguished from all other calls in the *G. liber* species complex by consisting of a single click-like note containing a low number of well-separated pulses (2–4 pulses/note), with distinct frequency bands recognizable up to 8500 Hz (see species accounts below for detailed call descriptions).

Calls from Marojejy Camp Simpona (lineage NE1) mainly differ from calls of NCE1 and possibly NCE2 by indistinct pulse structure, stronger amplitude modulation within notes and higher dominant frequency. No call recordings are available from lineage NE2.

Table 1. Measurements of specimens of the *Guibemantis liber* complex with reliable molecular identification (all in mm). For abbreviations of measurements, see Materials and Methods. Other abbreviations: HT, holotype; PT, paratype.

Field Number	ZSM Number	Status	Locality	Lineage	Sex	SVL	HW	HL	HTD	HED	END	NSD	NND	HAL	FORL	HIL	FOL	FOTL	TIBL
Guibemantis razoky **sp. nov.**																			
ZCMV 12515 (HT)	ZSM 1746/2010	HT	Bemanevika	NCENTR	male	28.0	9.3	10.7	3.1	4.4	2.8	1.7	2.5	8.7	17.4	43.3	13.6	20.2	14.0
ZCMV 12532	ZSM 1749/2010	PT	Bemanevika	NCENTR	male	30.2	9.7	11.4	3.1	4.2	2.8	2.0	2.5	9.4	17.9	49.0	14.4	21.4	15.2
ZCMV 12516	ZSM 1747/2010	PT	Bemanevika	NCENTR	male	26.5	8.3	10.2	2.1	4.0	2.6	1.4	2.6	8.2	15.5	43.0	13.3	19.6	14.5
ZCMV 12531	ZSM 1748/2010	PT	Bemanevika	NCENTR	male	31.5	10.0	12.0	2.8	4.4	3.0	2.3	2.4	9.2	19.6	49.2	15.5	22.2	16.0
ZCMV 12523	ZSM 1837/2010	PT	Bemanevika	NCENTR	male	29.8	10.0	11.0	2.5	4.6	2.9	1.9	2.2	9.0	17.3	45.5	13.4	19.8	14.5
FG/MV 2002-0874	ZSM 877/2003	PT	M. d'Ambre	NOR	male	32.4	10.8	11.6	3.0	4.3	2.7	2.2	2.4	10.0	20.4	51.4	15.5	22.7	15.7
FG/MV 2002-0875	ZSM 878/2003	PT	M. d'Ambre	NOR	male	33.9	11.2	12.7	2.8	4.4	4.0	2.2	2.3	10.3	22.6	51.8	16.1	23.0	16.5
MSZC 0520	ZSM 119/2018	PT	M. d'Ambre	NOR	male	33.6	11.4	12.2	2.4	4.1	4.0	2.2	2.8	9.7	21.7	51.9	15.1	22.6	15.9
FG/MV 2002-0898	ZSM 890/2003	PT	M. d'Ambre	NOR	male	29.3	9.7	10.9	2.4	4.1	2.6	1.9	1.8	9.0	19.8	43.8	13.1	20.8	14.5
ZCMV 12539	ZSM 1750/2010	PT	Bemanevika	NCENTR	female	31.7	9.6	11.4	2.6	4.4	3.0	1.8	2.4	9.8	19.6	47.5	14.4	20.8	15.7
ZCMV 12514	ZSM 1745/2010	PT	Bemanevika	NCENTR	female	29.8	9.7	11.2	3.0	4.4	2.5	1.7	2.3	8.8	17.6	45.5	13.8	20.3	14.7
FG/MV 2002-0899	ZSM 891/2003	PT	M. d'Ambre	NOR	female	32.8	10.8	12.0	2.4	4.0	3.2	2.0	2.9	10.9	21.0	45.2	15.0	22.3	15.2
Guibemantis liber																			
ZCMV 0791	ZSM 5083/2005	—	Andasibe	NCE1	male	28.9	9.3	10.8	2.4	4.0	2.5	2.0	2.4	8.5	18.3	43.9	13.3	20.0	13.4
ZCMV 8066	ZSM 1781/2008	—	Ambodisakoa	NCE2	male	27.0	8.9	10.6	2.2	3.7	2.6	2.0	2.2	8.6	16.8	41.1	12.3	17.9	13.5
ZCMV 8076	ZSM 1783/2008	—	Ambodisakoa	NCE2	male	26.4	8.0	9.7	2.2	3.3	2.5	1.6	2.0	7.2	15.0	41.0	10.8	17.9	13.3
ZCMV 8088	ZSM 1785/2008	—	Ambodisakoa	NCE2	male	23.7	7.3	8.8	2.0	3.8	2.5	1.8	2.9	6.2	13.2	36.9	11.3	15.3	12.0
ZCMV 3199	ZSM 491/2006	—	Ranomafana	SCE	male	27.1	8.4	9.3	2.4	3.3	2.3	1.7	2.2	8.9	18.4	42.0	13.3	18.5	13.0
ZCMV 3028	ZSM 487/2006	—	Ranomafana	SCE	male	24.2	7.9	8.7	2.1	3.5	2.4	1.7	2.3	8.0	17.5	38.4	11.5	17.4	12.0
ZCMV 3041	ZSM 488/2006	—	Ranomafana	SCE	male	26.3	8.9	9.9	2.4	3.6	3.2	1.8	2.5	8.0	18.0	39.9	12.1	17.7	12.3
ZCMV 3018	ZSM 483/2006	—	Ranomafana	SCE	male	25.2	8.0	10.0	2.1	3.5	3.2	1.7	2.9	8.4	17.5	36.5	12.2	18.1	12.4
ZCMV 5833	ZSM 2336/2007	—	Manombo	SOE	male	27.8	9.3	10.7	2.7	4.2	2.6	2.0	2.4	8.0	18.5	40.6	12.5	18.2	13.0
ZCMV 5830	ZSM 2335/2007	—	Manombo	SOE	male	25.7	8.1	9.8	2.1	3.4	2.9	1.1	2.1	6.9	16.0	40.7	11.8	17.9	12.3
FGZC 4452	ZSM 477/2010	—	Anosibe An'Ala	NCE1	female	24.4	8.3	10.0	2.7	4.0	2.6	1.7	2.3	8.0	16.2	40.6	12.9	18.8	13.0
ZCMV 0786	ZSM 5082/2005	—	Andasibe	NCE1	female	30.7	10.3	10.9	2.9	4.5	2.8	2.0	2.2	8.4	18.0	44.4	13.8	19.5	14.0
ZCMV 1074	ZSM 2329/2007	—	Andasibe	NCE1	female	26.8	8.5	9.7	2.6	3.7	2.8	2.0	2.3	8.2	18.3	43.9	13.4	19.2	13.5
ZCMV 5675	ZSM 2332/2007	—	Ambohitantely	NCE2	female	33.3	11.7	13.1	2.3	4.6	3.7	2.4	2.7	10.5	21.3	51.2	15.7	23.4	17.0
ZCMV 5672	ZSM 2331/2007	—	Ambohitantely	NCE2	female	32.1	9.9	12.2	2.4	4.0	3.4	2.0	2.3	9.7	20.8	49.4	15.1	22.4	15.8
ZCMV 3021	ZSM 485/2006	—	Ranomafana	SCE	female	27.0	8.0	10.0	2.2	3.5	3.5	1.9	2.4	8.9	18.0	41.4	13.3	19.0	13.1
Guibemantis razandry **sp. nov.**																			
FGZC 2851 (HT)	ZSM 293/2005	HT	Marojejy, Camp Simpona	NE1	male	25.6	8.3	9.7	2.7	3.9	2.7	1.6	2.7	7.8	18.0	42.1	12.5	19.9	13.6
FGZC 2865	ZSM 294/2005	PT	Marojejy, Camp Simpona	NE1	male	25.9	9.0	10.2	2.2	4.0	2.9	1.6	2.5	8.4	18.4	42.0	13.0	20.4	14.0

Field Number	ZSM Number	Status	Locality	Lineage	Sex	SVL	HW	HL	HTD	HED	END	NSD	NND	HAL	FORL	HIL	FOL	FOTL	TIBL
FGZC 2867	ZSM 295/2005	PT	Marojejy, Camp Simpona	NE1	male	24.4	7.8	9.2	2.0	3.9	2.6	1.4	2.6	8.2	16.5	41.0	12.5	18.3	12.7
ZCMV 15176	ZSM 424/2016	PT	Marojejy, Camp Simpona	NE1	male	27.4	8.7	10.3	2.2	3.7	2.6	1.5	2.4	8.6	18.9	44.2	12.5	18.2	13.6
ZCMV 12570	ZSM 1683/2010	PT	Bemanevika	NE1	male?	21.8	6.7	8.8	2.1	3.0	1.8	1.2	1.6	6.6	14.0	36.4	10.1	16.9	11.4
ZCMV 12584	ZSM 1689/2010	PT	Bemanevika	NE1	male?	22.7	7.2	9.0	2.8	3.4	2.2	1.5	2.2	7.0	14.0	33.2	10.1	16.3	10.8
ZCMV 12578	ZSM 1684/2010	PT	Bemanevika	NE1	male?	24.3	7.2	9.0	2.5	3.8	2.2	1.8	2.7	6.8	14.1	35.6	10.7	16.1	11.7
ZCMV 12579	ZSM 1685/2010	PT	Bemanevika	NE1	male?	23.8	7.2	9.0	3.0	3.3	2.3	1.4	2.8	6.4	13.9	36.9	11.4	16.4	11.4
ZCMV 12580	ZSM 1686/2010	PT	Bemanevika	NE1	male?	22.2	6.8	8.1	2.5	3.5	2.1	1.6	1.8	6.8	13.5	34.7	11.3	16.3	10.8
ZCMV 12581	ZSM 1687/2010	PT	Bemanevika	NE1	male?	23.1	7.3	8.5	2.6	3.7	2.0	1.7	2.4	6.9	14.8	35.6	10.7	16.2	11.0
ZCMV 12583	ZSM 1688/2010	PT	Bemanevika	NE1	male?	22.9	7.3	8.8	2.8	3.1	1.8	1.6	1.5	6.4	14.4	34.5	11.4	16.6	10.6
ZCMV 11218	ZSM 513/2009	PT	Makira	NE1	female	22.8	7.2	8.4	2.2	3.3	2.3	1.7	2.6	6.8	14.0	35.6	10.2	15.6	11.6
Guibemantis fotsitenda **sp. nov.**																			
FGZC 2781	ZSM 292/2005	HT	Marojejy, Camp Mantella	NE2	male	25.5	8.2	10.0	2.4	4.0	2.8	2.2	19.0	7.5	15.6	42.5	12.1	18.5	12.6
FGZC 2722	ZSM 290/2005	PT	Andrakata-Andapa	NE2	male	25.9	8.4	10.2	2.5	3.4	2.7	1.7	2.2	8.3	15.8	38.1	12.0	18.0	11.1
FGZC 2721	ZSM 289/2005	PT	Andrakata-Andapa	NE2	male	26.0	7.9	9.4	2.0	3.8	3.2	1.7	2.2	7.7	14.6	39.3	11.7	18.1	12.4
FGZC 2780	ZSM 291/2005	PT	Marojejy, Camp Mantella	NE2	male	25.1	8.3	9.7	2.7	4.0	2.9	1.7	2.3	7.5	15.7	40.5	11.6	18.2	12.7

In summary, even if not fully conclusive given the limitations mentioned above, bioacoustics provides additional evidence for the divergence of several of the genetic lineages in the *G. liber* complex.

Available nomina in the *Guibemantis liber* complex

Rhacophorus liber Peracca, 1893

This is the oldest nomen in the *G. liber* complex and was coined based on a series of 15 syntypes. The entire collection of amphibians and reptiles described by Peracca (1893) in the same paper came either from the surroundings of Andrangoloaka, or from the nearby Umbi valley ("dai dintorni di Andrangoloaka e dalla vicina valle dell'Umbi"). In the original description, Peracca (1893) provides measurements of four males (SVL 24–25.5 mm) and four females (SVL 27.5–30.5 mm). Guibé (1978) reported "M.H.N.P. 1894-3 et B.M. 1947.2.8.64-67" as "paratypes" of the species. Blommers-Schlösser (1979) examined two of the specimens mentioned by Guibé (1978), i.e., the female specimens BMNH 1947.2.8.64, and MNHN 1894.3, plus one additional specimen (BMNH 1947.2.8.63), all purportedly original syntypes of *G. liber*, and designated the latter of these (BMNH 1947.2.8.63) as lectotype. Blommers-Schlösser and Blanc (1991) report these same three numbers as type series. Gavetti and Andreone (1993) listed 13 specimens under the number MZUT An86 as paralectotypes. Summarizing this information, there would be either two or four specimens of the original syntype series in the NHMUK (= BMNH) in London (BMNH 1947.2.8.63–64 according to Blommers-Schlösser 1979, or 1947.2.8.64–67 according to Guibé 1978), one specimen in Paris (MNHN 1894.3), and 13 specimens in Turin (MZUT An64 – merged by Gavetti and Andreone 1993 from two series comprising 8 and 5 specimens, respectively), summing up to more than the original 15 syntypes reported by Peracca. As already suspected by Gavetti and Andreone (1993), it therefore is likely that some of these specimens were not part of the original syntype series.

However, there is at present no reason to doubt that the lectotype designated by Blommers-Schlösser (1979), BMNH 1947.2.8.63, corresponds to one of the original syntypes. Furthermore, this author also provided measurements of the lectotype and two paralectotypes whose relative hand length (HAL/SVL 0.32–0.34) matches the range typically observed in other individuals assigned to this species (and is larger than in several other *Pandanusicola*: Blommers-Schlösser 1979). Lehtinen et al. (2012) provided a photograph of the preserved lectotype, as well as newly taken measurements of it and of the paralectotype BMNH 1947.2.27.64 confirming the generally large hands of these individuals (HAL/SVL 0.31–0.32). Also, one of the paralectotypes (MZUT An86.1) figured by Gavetti and Andreone (1993) matches very well the general appearance of other individuals typically assigned to this species. Despite some uncertainties about the compo-

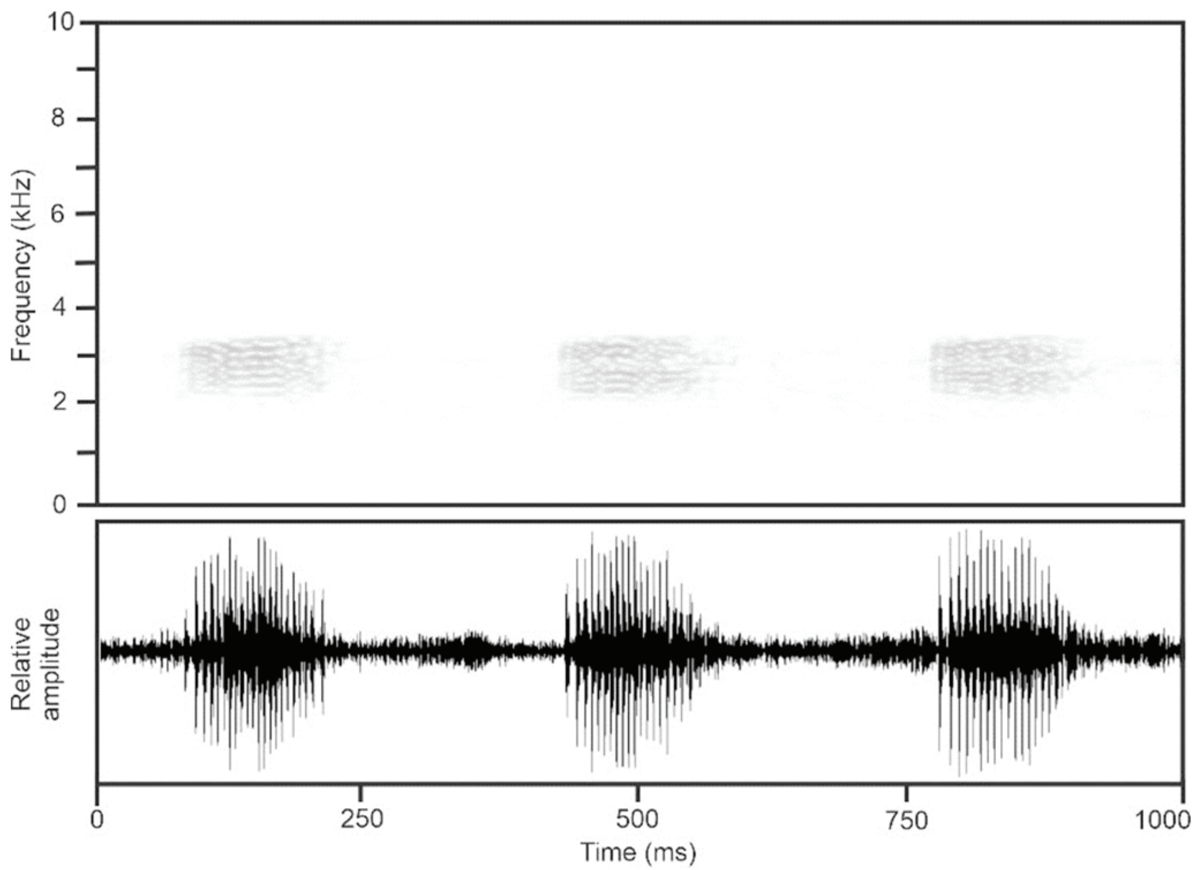

Figure 14. Audiospectrogram and corresponding oscillogram of a series of advertisement calls (3 calls) of *Guibemantis liber* (clade NCE1) recorded on 8 February 2000 at Mandraka. Recording band-pass filtered at 1500–4000 Hz.

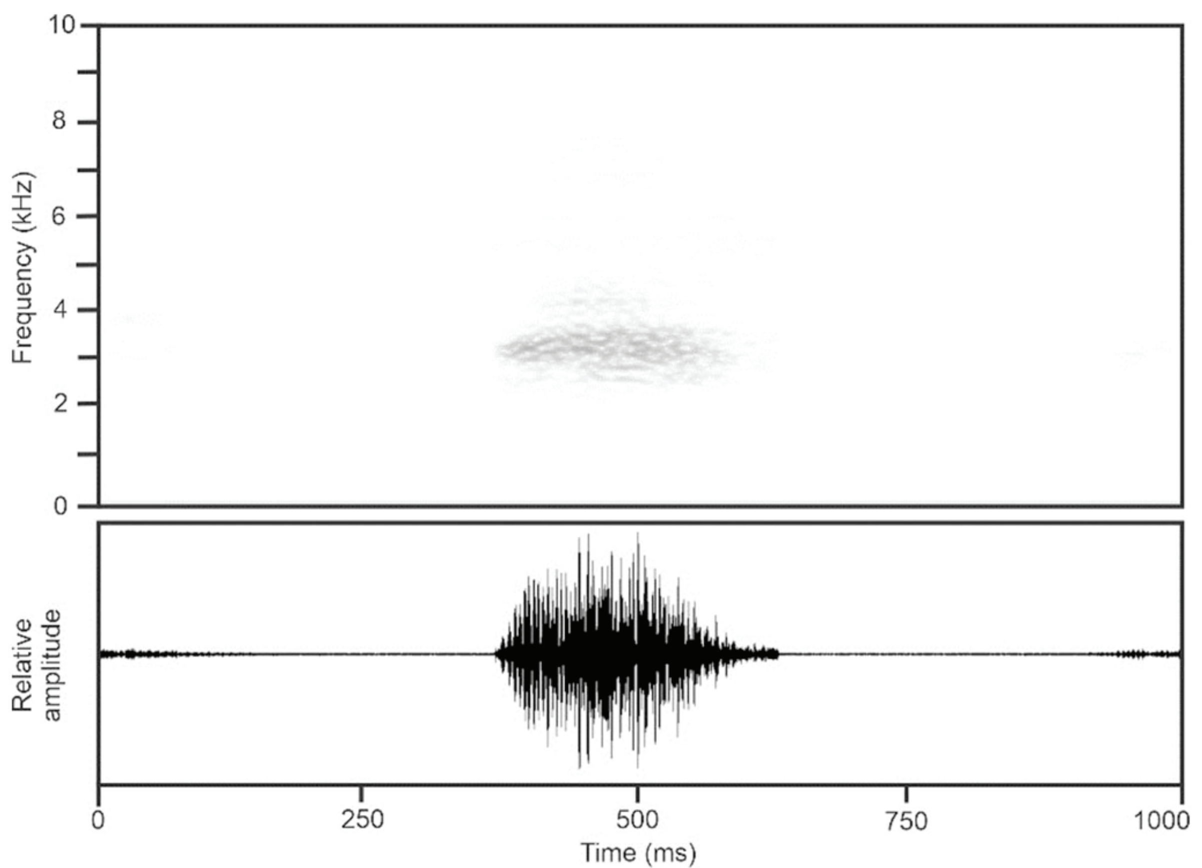

Figure 15. Audiospectrogram and corresponding oscillogram of one advertisement call of *Guibemantis liber* (clade NCE2) recorded on 12 February 2008 at Ambodisakoa. Recording band-pass filtered at 1500–8000 Hz.

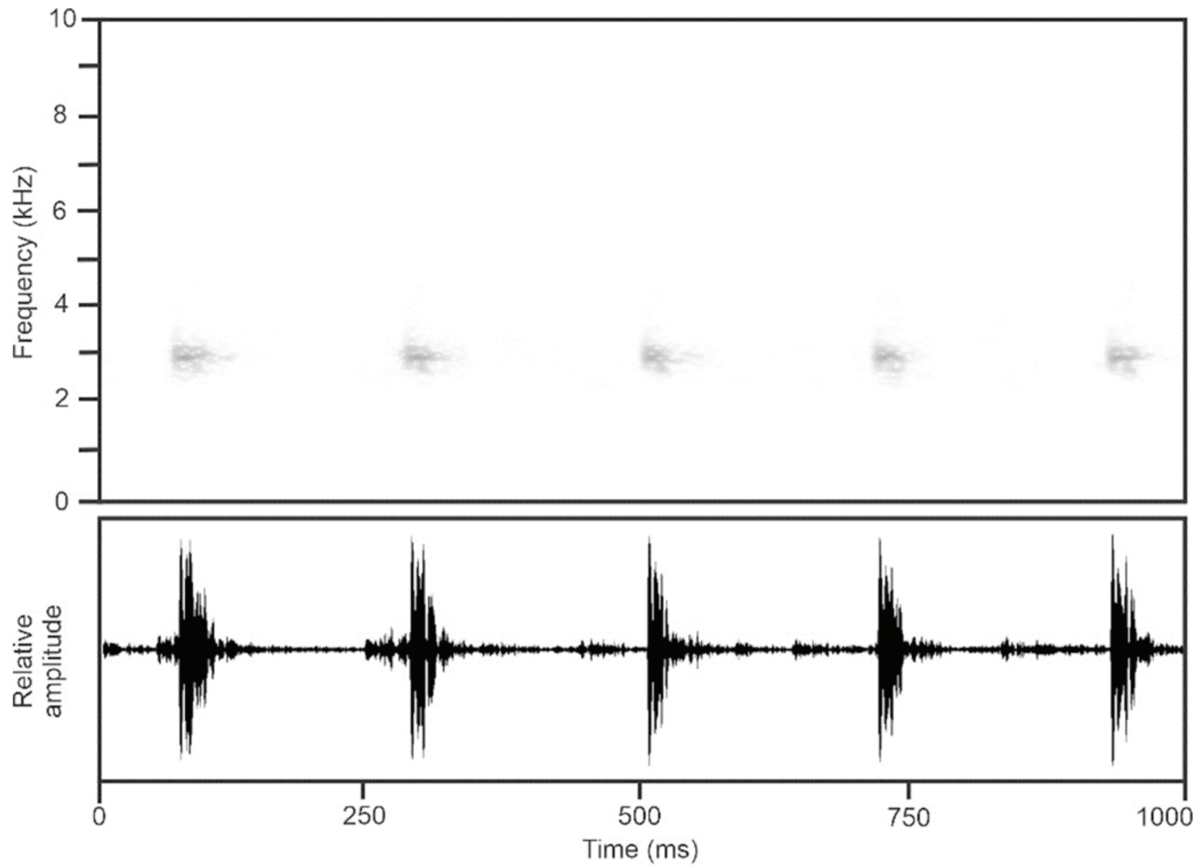

Figure 16. Audiospectrogram and corresponding oscillogram of a series of advertisement calls (5 calls) of *Guibemantis liber* (clade SCE) recorded on 20 January 2004 near Vohiparara (Ranomafana area). Recording band-pass filtered at 2400–5100 Hz.

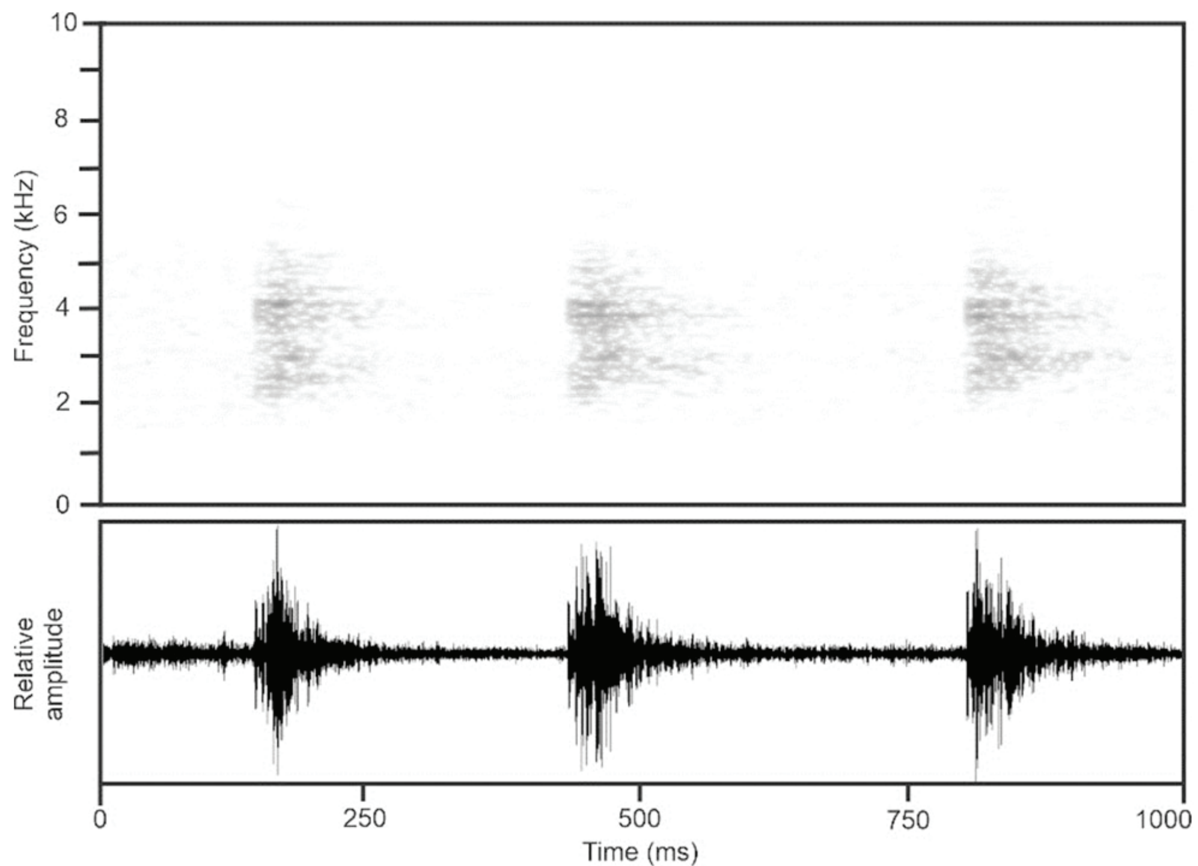

Figure 17. Audiospectrogram and corresponding oscillogram of a series of advertisement calls (3 calls) of *Guibemantis razandry* **sp. nov.** recorded on 16 February 2005 at Marojejy National Park. Recording band-pass filtered at 1000–9500 Hz.

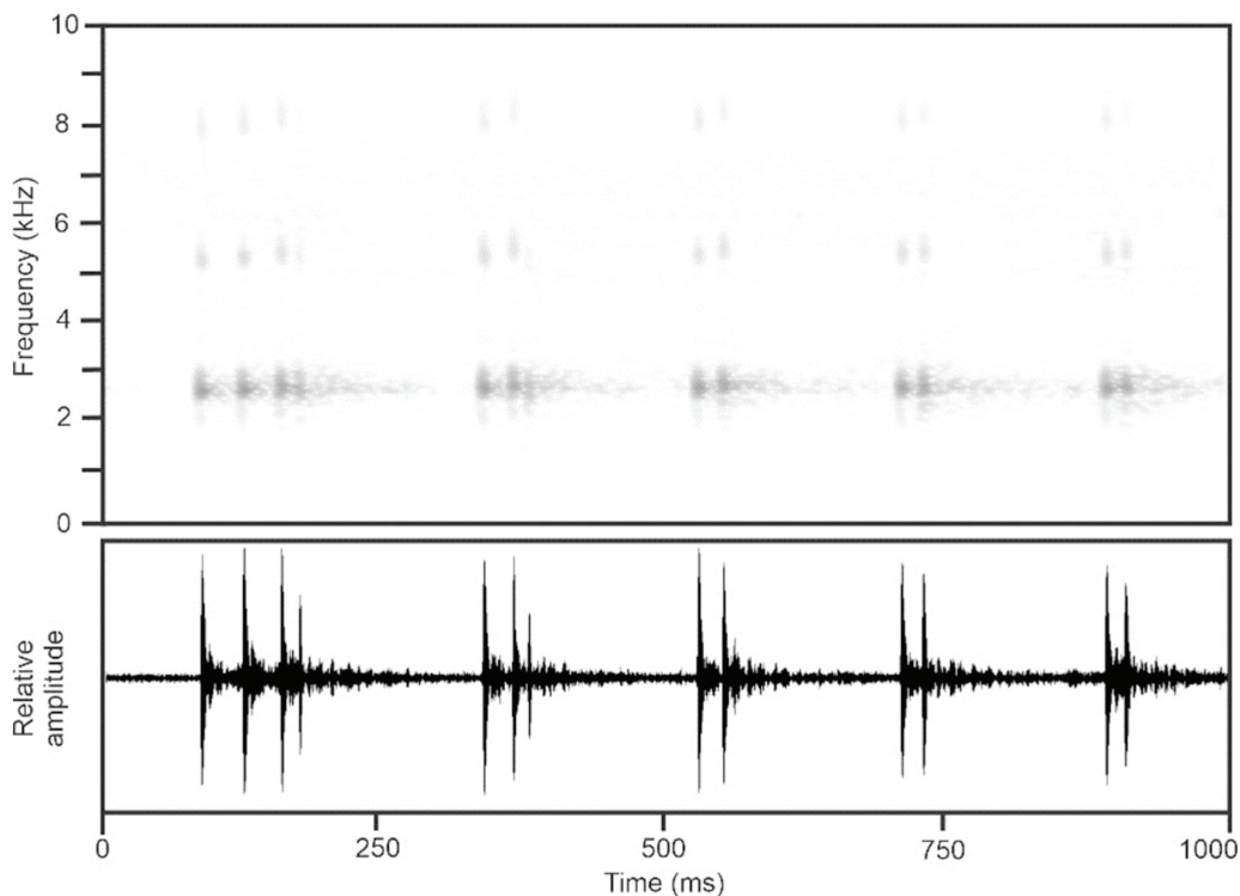

Figure 18. Audiospectrogram and corresponding oscillogram of a series of advertisement calls (5 calls) of *Guibemantis razoky* **sp. nov.** recorded on 14 March 1994 at Montagne d'Ambre National Park. Recording band-pass filtered at 1000–9500 Hz.

sition of the type series, it therefore seems clear that the nomen *Rhacophorus liber* Peracca, 1893 indeed applies to frogs herein considered as part of the *G. liber* complex, and based on geographical considerations, most likely to lineage NCE1. The body size of the types as well as their origin from Andrangoloaka in the Northern Central East allow us to exclude the possibility that this name would be applicable to any of the lineages of the complex occurring in northern Madagascar.

Gephyromantis albogularis Guibé, 1947

This nomen is based on a holotype specimen, which according to the original description is an adult male of a purported SVL of 27 mm, "no. 8.146" (Guibé 1947). Additionally, one paratype was designated in the original description, a male of 25 mm with the "no 8.147", in the "Muséum National d'Histoire Naturelle de Paris". According to the original description, the species has a "coloration blanche immaculée de la gorge", which perfectly matches the typical pattern in breeding males of *G. liber*. The specimens originated from "Madagascar, sans précision de localité, acquis de M. Frantz[sic] Sikora (1891)". The name-bearing type is thus the holotype MNHN 8146 as reported in Blommers-Schlösser and Blanc (1991). Measurements of the paratype MNHN 8147 (SVL 28.0 mm) were given in Blommers-Schlösser (1979).

Although there is no precise type locality for *albogularis*, it is likely that the types originated from central eastern Madagascar or perhaps from south-eastern Madagascar. Franz Sikora spent seven or eight years in Madagascar, first in Antananarivo, and later (probably 1899) in Fort Dauphin (Tolagnaro), and visited the mission in Andrangoloaka (Dorr 1997). Other amphibian and reptile type material collected by Sikora originated from these sites, from Toliara in the arid South West, or from Anevoka in the Northern Central East. We are not aware of any material collected or provided by Sikora from northern Madagascar, suggesting that *albogularis* cannot be an earlier available name for any of the three new species named herein. However, if any of the genetic lineages of *G. liber* from the Northern Central East or Southern Central East were to be considered as separate subspecies or species, the name *albogularis* needs to be considered as a possible earlier name for one of them.

It is possible that the type material of *albogularis* and of *liber* (also collected by Sikora) was part of the same collection, in which case *albogularis* would also originate from Andrangoloaka or a nearby site; however, the type material of *albogularis* was apparently obtained by the Paris museum from Sikora in 1891, and the material of *liber* by the Turin museum in 1893 (Gavetti and Andreone 1993). Lehtinen et al. (2011) also argued for synonymy of *albogularis* with *liber*, based on examination of holotype and paratype which in 2010 were in an extreme-

ly poor state of preservation. SVL was reported to be 23.8 mm in the holotype (a typographic mistake for 28.3 mm), and its HAL/SVL ratio of 0.33 was congruent with the values found in *G. liber.*

Gephyromantis variabilis Millot and Guibé, 1951

This nomen was coined by Millot and Guibé (1951) based on 28 syntypes, according to the original description: 8 males and 11 females from Perinet (= Andasibe), probably collected in August 1949 (VIII/49) and 3 males and 6 females from Itremo, collected September 1949 (IX/49), in *Pandanus* plants, all deposited in the "Muséum d'Histoire naturelle de Paris".

Unfortunately, the labelling and identification of these syntypes in the MNHN collection is quite confusing, and in addition, it is necessary to deal with an invalid lectotype designation by implication. Guibé (1978) listed only one catalogue number for the type material, as follows: "Forêt de Perinet et Itremo. Syntypes M.H.N.P. 1953-116". Blommers-Schlösser (1979) then considered MNHP 1953-116 as "the holotype of *Gephyromantis variabilis*" without mentioning any other specimens, and Blommers-Schlösser and Blanc (1991) wrote regarding the type series of *G. variabilis*, without any further comment or justification: "Périnet. Coll. Millot. Holotype M.H.N.P. 1953-116. Paratypes M.H.N.P.1953-117-119. Itremo." This information has been considered since to represent a "lectotype designation by implication" (Frost 2021). However, Article 74.5 of the International Code of Zoological Nomenclature (ICZN 1999) states unambiguously for lectotype designations before 2000, that "When the original work reveals that the taxon had been based on more than one specimen, a subsequent use of the term 'holotype' does not constitute a valid lectotype designation unless the author, when wrongly using that term, explicitly indicated that he or she was selecting from the type series that particular specimen to serve as the name-bearing type." The work of Blommers-Schlösser and Blanc (1991) does not contain any such explicit statement and therefore, at present no properly designated lectotype for *G. variabilis* exists. Furthermore, as argued in the following, it is even uncertain whether the individual currently labelled MNHN 1953.116 was part of the original syntype series.

We examined the type material and associated specimens in the Paris museum in 2010. According to the original catalogue of the Paris museum, two catalogue numbers are considered to be types of *variabilis*: MNHN 1953.116 and MNHN 1953.117. The former number, MNHN 1953.116, according to the catalogue refers to one specimen from Morafenobe (and not Perinet as stated by Blommers-Schlösser and Blanc 1991), whereas MNHN 1953.117 according to the catalogue refers to 8 specimens from Itremo. If the provenance of MNHN 1953.116 from "Morafenobe" is correct, then it likely does not belong to the original syntype series of *G. variabilis* which according to the original description was composed of only specimens from Perinet (= Andasibe) and Itremo, even if the catalogue number of this specimen agrees with that given for the syntype series in the original description. Furthermore, the specimen labelled MNHN 1953.116 is a small juvenile specimen with completely faded color pattern, and with relatively short hands suggesting it belongs to another species of *Pandanus*-dwelling *Guibemantis*. To make things more complicated, the series MNHN 1953.117 originally consisted of several specimens, while it is unclear whether this may also apply to MNHN 1953.116.

Upon examination of the specimens (stored in two separate jars), we found that the same jar containing MNHN 1953.116 also contained the specimens MNHN 1953.111 from Ambila, MNHN 1953.112 from Tsaratanana (3 specimens according to catalogue), MNHN 1953.114 from Perinet ("Pandanus, mousses des arbres 1949"), MNHN 1953.115 with same data as MNHN 1953.114 (5 specimens), and also 1953.118–119 from Perinet. All these numbers refer to specimens of *Guibemantis*, but not explicitly marked as types of *variabilis* on their labels or in the catalogue. The jar in which MNHN 1953.117 was preserved, also contained MNHN 1953.113 from Perinet ("nomb." according to the catalogue, probably meaning "nombreuses" = many specimens), plus numerous additional specimens bearing numbers from other years than 1953.

For each of the numbers given above (except for MNHN 1953.116), we found in the examined jars single specimens only, although the catalogue in part specifies the existence of multiple specimens under that number. In subsequent catalogue entries we found information that these additional specimens apparently had been relabeled: MNHN 1953.115, specimens relabeled as MNHN 1975.828–830; MNHN 1953.111, specimens relabeled as MNHN 1975.835–851; MNHN 1953.112, specimens relabeled as MNHN 1975.880–881; MNHN 1953.117, specimens relabeled as MNHN 1975.930–935; MNHN 1953.118, specimens relabeled as MNHN 1975.936–948.

Of these specimens, MNHN 1953.111–112 by superficial examination of color patterns might belong to species of *Pandanusicola* different from *G. liber*, MNHN 1953.112 being an adult male recognizable by well-developed femoral glands. Specimen MNHN 1953.118 is clearly an adult male of *G. liber*, as are MNHN 1953.114–115, both recognizable by their white throats. In MNHN 1953.119 the color pattern is largely faded, but as can be assumed from its rather large hands, it most likely represents *G. liber* as well. MNHN 1953.117 is an adult female of *G. liber* as clearly recognizable by its large hands and typical color pattern. Of the relabeled specimens, MNHN 1975.930–934 can also be identified as *G. liber* based on their large hands.

Because currently no validly designated lectotype of *G. variabilis* exists and for several of the specimens discussed above it is not fully clear whether they belong to the original syntype series of this nomen, we here designate MNHN 1953.117 as lectotype of *Gephyromantis variabilis* Millot and Guibé, 1951. This specimen is listed as type in the MNHN catalogue and as "paratype" in Blommers-Schlösser and Blanc (1991), and its prove-

nance Itremo agrees with the collecting locality of part of the syntype series in the original description. We do not designate a specimen from Perinet (= Andasibe) as lectotype because the identity of the various Perinet specimens discussed above is less clear, and furthermore, because Andasibe seems to be located in the contact zone of two main lineages of *G. liber* (NCE1 and NCE2) which could create future taxonomic issues if these lineages were to be distinguished at the subspecies or species level. As stated above, the lectotype specimen can be assigned based on its large hands and typical color pattern to *G. liber*, and the nomen *variabilis* is therefore stabilized as junior synonym of this species. The type locality of *G. variabilis* thus becomes restricted to Itremo. A photograph of the preserved lectotype (photographed in 2010) is shown in Fig. S4. Measurements of the lectotype taken by us in 2010 are as follows (all in mm): SVL, 26.0; HW, 9.1; HL, 10.3; ED, 3.3; TD, 1.9; END, 2.5; NSD, 1.8; NND, 2.4; HAL, 8.4; FORL, 18.3; HIL, 43.7; FOTL, 19.8; FOL, 12.6; TIBL, 13.7.

Taxonomic conclusions

The combined evidence from mitochondrial and nuclear-encoded genes, geographical distribution, morphology and bioacoustics provides conclusive evidence that *Guibemantis liber* as currently understood is a complex of multiple species. The most obvious evidence for this conclusion is the syntopic occurrence, without genetic admixture and under maintenance of morphological differences, of lineages NE1 and NCENTR at various localities (Bemanevika, Makira, and Tsaratanana). We here propose an initial though still incomplete taxonomic resolution of the *G. liber* complex, as follows:

(1) Based on geographic and morphological arguments, we conclude that the type material of *G. liber*, and of its junior synonym *albogularis*, likely belong either to lineage NCE1 or NCE2. Through the lectotype designation herein, *variabilis* is stabilized as junior synonym of *G. liber* as well; its assignment to a lineage (likely NCE1 or SCE) remains pending, but based on geographical arguments we can exclude that this nomen applies to any of the northern lineages.

(2) Lineages NE1 and NE2 are most divergent within the *G. liber* complex; they are sister to all other lineages in the complex (and may fall outside it) and differ by small body size and some details of color pattern. NE1 occurs syntopically with NCENTR over a wide area without admixture (haplotype sharing only in POMC) and thus clearly deserves species status.

(3) The two northeastern lineages, NE1 and NE2, also occur sympatrically at Marojejy in very close geographical proximity but apparently separated by elevation. Due to this almost-sympatric occurrence without any signs of admixture, combined with divergence in mitochondrial genes (2.8–3.9% in 16S) and in nuclear encoded genes (long branches in the phylogenomic analysis) between NE1 and NE2, we consider each of them to be a distinct species.

(4) Lineage NCENTR+NOR differ from the lineages occurring in Madagascar's central east by a considerable mitochondrial divergence, large body size, and advertisement calls. The phylogenomic analysis based on concatenated FrogCap data places this lineage sister to the remaining *G. liber* lineages, although this position was neither recovered by the cytochrome *b* tree (Fig. 1), nor the multigene tree (Fig. 7; probably reflecting the mainly mitochondrial signal in the multigene data set). We consider both NCENTR+NOR together to form one separate species-level lineage.

(5) Among the lineages distributed in Madagascar's Northern and Southern Central East (NCE1, NCE2, NCC, SCE, SOE), several are characterized by strong mitochondrial divergence, limited sharing of nuclear-encoded alleles, and possibly bioacoustic differences. In particular, the southern lineages SCE+SOE have only limited sharing of alleles with the NCE1+NCE2+NCC lineages in several of the nuclear-encoded genes studied herein. It is likely that additional partitioning of *G. liber* into species or subspecies is warranted, but here we refrain from further taxonomic changes due to incomplete knowledge on the lineage assignment of the various earlier available names (in particular *variabilis*), lack of high-quality call recordings from genotyped males, limited amount of morphological data from genotyped specimens, and sampling gaps. For a more detailed assessment of the data needed for further taxonomic resolution of this complex, see Discussion below.

Based on this rationale, we consider the current evidence to be sufficient to scientifically name lineages NE1, NE2 and NCENTR+NOR as three new species in the *G. liber* complex.

Descriptions of three new species

Guibemantis razandry sp. nov.

https://zoobank.org/2D2B88BB-1096-4381-ADE3-2850FC472417

Figs 9, 11

Holotype. ZSM 293/2005 (field number FGZC 2851), adult male, collected in Marojejy National Park (14.43767°S, 49.77555°E, 1326 m a.s.l.), Sava Region, northeastern Madagascar on 26 February 2005 by F. Glaw, M. Vences and R.D. Randrianiaina.

Paratypes. A total of 26 specimens: ZSM 294–295/2005 (field numbers FGZC 2865, FGZC 2867), two adult males, same collection data as holotype; ZSM 424/2016 (field

number ZCMV 15176), adult male, collected at Marojejy, near Camp 3 "Simpona" (14.43661°S, 49.74335°E, 1325 m a.s.l.) on 17 November 2016 by M.D. Scherz, A. Rakotoarison, M.C. Bletz, M. Vences and J. Razafindraibe; ZSM 513/2009 (ZCMV 11218), adult male, collected near Hevirina, western slope of Makira Reserve (ca. 15.4490°S, 49.1119°E, 1093 m a.s.l.), on 23 June 2009 by M. Vences, D.R. Vieites, F. Ratsoavina, R.D. Randrianiaina, E. Rajeriarison, T. Rajofiarison, and J. Patton; ZSM 1682–1689/2010 (field numbers ZCMV 12569–12584), adults and subadults, collected near Bemanevika river (14.48251°S, 48.62723°E, 1109 m a.s.l.) on 29 June 2010 by M. Vences, D. Vieites, R.D. Randrianiaina, F. Ratsoavina, S. Rasamison, A. Rakotoarison, E. Rajeriarison and T. Rajoafiarison; ZSM 1738–1743/2010 (field numbers ZCMV 12377, 12441, 12462, 12463, 12466, 12468), adults and subadults collected at Camp 2 (Matsaborimaika) on the Tsaratanana Massif (14.15256°S, 48.95728°E, 2021 m a.s.l.) on 15–20 June 2010 by M. Vences, D. Vieites, R.D. Randrianiaina, F. Ratsoavina, S. Rasamison, A. Rakotoarison, E. Rajeriarison and T. Rajoafiarison; ZSM 1894–1900/2009 (ZCMV 11352–11365), from the western slope of Makira Reserve (probably from Ampofoko campsite), collected in June/July 2009 by M. Vences, D.R. Vieites, J. Patton, R.D. Randrianiaina, F. Ratsoavina and E. Rajeriarison; KU 347374 (CRH1693), specimen of unknown sex and maturity, collected at Anjanaharibe-Sud Special Reserve (14.698°S, 49.465°E) by C.R. Hutter and Z.F. Andriampenomanana.

Diagnosis. This species corresponds to the mitochondrial lineage NE1 as defined herein, and to the candidate species *Guibemantis* sp. Ca21 according to Perl et al. (2014). It is assigned to the subgenus *Pandanusicola* of the genus *Guibemantis* based on presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), small body size, moderate to weakly expressed webbing between toes, connected lateral metatarsalia, the presence of both inner and outer metatarsal tubercles, femoral glands in males, absence of nuptial pads, small body size (SVL 24.4–25.9 mm in reliably sexed males and 22.8 mm in one female), and molecular phylogenetic relationships. Within *Pandanusicola*, the new species is distinguished from all species except *G. liber* and *G. tasifotsy* by femoral glands type 1 (vs. type 2) as defined by Glaw et al. (2000), thus possessing many small gland granules in a relatively diffuse field covering most of the thigh ventrally, and by its probable breeding in open swamps (vs. phytotelmic breeding in *Pandanus* leaf axils). It can be distinguished from *G. tasifotsy* by its different brownish color pattern lacking a green dorsal and lateral coloration with a series of distinct white blotches along the lower flanks and its strongly different advertisement call, consisting of a pulsatile note with numerous pulses being largely fused (vs. a trill-like note containing 3–7 distinctly separated pulses). The new species differs from all *G. liber* lineages occurring in the Northern and Southern Central East of Madagascar by its high DNA divergence, with > 5% uncorrected pairwise distance in the mitochondrial 16S gene and 20 diagnostic positions in the analyzed fragment of the cytochrome *b* gene (see Appendix 2 for a list of diagnostic sites), as well as probably by a somewhat smaller snout-vent length. For a distinction from the other two new species described herein, see below.

Description of holotype. Adult male in excellent state of preservation (Fig. 9). A small piece of muscle tissue from right thigh removed for molecular analysis. SVL 25.6 mm. For full morphometric measurements see Table 1. Body relatively slender; head slightly longer than wide, wider than body; snout rounded in dorsal, ventral, and lateral views; nostrils much nearer to tip of snout than to eye and pointed anterolaterally; canthus rostralis distinct, slightly concave; loreal region concave; tympanum distinct, relatively small, its diameter 69% of eye diameter; distinct supratympanic fold; tongue ovoid, distinctly bifid posteriorly; vomerine teeth as one weakly expressed rounded aggregation posterolateral of each choana; choanae small, rounded. Forelimbs slender; subarticular tubercles distinct and single; central metacarpal tubercle large and rounded, outer metacarpal tubercle smaller and oval; a small but indistinct prepollex (which could also be considered as an inner metacarpal tubercle) at base of first finger. Fingers without webbing; relative finger length I<II<IV<III; finger discs distinctly enlarged; nuptial pads absent. Outer toe and finger discs darker than inner toe and finger discs. Hind limbs long and slender; when adpressed along body, tibiotarsal articulation reaches beyond eye; lateral metatarsalia connected by tissue; inner metatarsal tubercle distinct, larger than outer; outer metatarsal tubercle distinct; webbing formula of foot 1(traces), 2i(traces), 2e(1), 3i(2.5), 3e(1.5), 4i(2.75), 4e(3), 5(1); relative toe length I<II<III = V<IV. Skin dorsally smooth; ventral skin smooth on throat and chest, slightly granular on belly. Femoral glands relatively distinct from external view, consisting of large number of small gland granules in a relatively diffuse field covering most of thigh ventrally, thus of type 1 as defined by Glaw et al. (2000). In life gland granules distinctly recognizable as small greenish-yellowish units, at least 170 in one gland (Fig. 11).

After sixteen years in preservative (70% EtOH; Fig. 9), dorsal background coloration light brownish with two prominent dark brownish dorsolateral bands extending posteriorly from eye orbits to hips. Rostral stripe dark brownish. Dorsally numerous dark reddish brown irregular spots present, particularly between orbits and at middorsum. Forelimbs have irregular and partially interrupted dark, brownish bands and spots extending from shoulders to fingers. Outer finger discs reddishbrown. Dorsal surface of thigh with broad dark blotches and interrupted bands. These darkish patterns extend to shanks and continue as single blotches and spots on feet and toes. Like finger discs, some outer toe discs dark reddish brown, noticeably different than adjacent tissue.

Based on photographs of holotype in life (Fig. 11), body coloration was as follows: on dorsum reddish brown, on limbs and laterally greenish gray. Dorsolaterally a thin yellowish line on each side. Irregular-sized dark blackish brown spots and dots middorsally and later-

ally, but particularly on both fore- and hindlimbs. Supratympanic fold and rostral stripe blackish brown. Paired, thin white dorsal lines in life (not visible in preservative) (Fig. 11). Background color of ventral surface whitish to greenish, chest and throat bright white. Posterior and lateral parts of abdomen semi-transparent. Femoral gland granules yellow. Iris (whitish in preservative) apparently glossy golden in life.

Variation. Specimens of *G. razandry* show a high variation in color pattern, but overall appear to have a lighter color compared to the sympatric *G. razoky* **sp. nov.** (described below; compare Fig. 11 vs. Fig. 12). In general, the ground color of *G. razandry* **sp. nov.** is light brown to beige, with different darker brown patterns. ZSM 513/2009 has a light brown ground color, darker flanks, and a broad light beige vertebral stripe. ZSM 424/2016 has an extremely contrasted pattern, with a light beige dorsal side, bordered by dark brown color that occupies most of the flanks. Breeding males (such as ZSM 424/2016) have bright white vocal sacs, but these are not visible in other individuals collected out of the breeding season and some of these could in fact not be sexed with full reliability (as part of the inner organs have been damaged during dissection for amphibian parasites). For variation in morphometric features see Table 1.

Natural history. Males of *G. razandry* have been observed at night, calling from perches in the vegetation about 1–2 m above the ground, typically at the edge of swamps in primary rainforest. On the Marojejy Massif, they sometimes call sitting on leaves in the vegetation near dry beds of temporary headwater streams. At the same site, we also found a clutch with quite well-developed larvae on a leaf that may belong to this species. We also found clutches that were faded and whitish, which may have been infected with a fungus or bacterial growth. Outside of the breeding season (in June) we found specimens near Bemanevika River hidden in the leaf axils of *Pandanus* screw pines, syntopic with *Blommersia blommersae*, *Guibemantis* sp. aff. *pulcher*, and *G. razoky* **sp. nov.** The species occurs both in areas of primary rainforest, and in highly degraded and fragmented forest patches, e.g., near Bemanevika.

Vocalization. Advertisement calls recorded on 16 February 2005 at Marojejy National Park (air temperature unknown) consist of a single pulsatile note of somewhat variable duration. Calls (= notes) are usually emitted in short series at rather regular intervals (Fig. 17). No clear pulse structure is evident within notes, with pulses being largely fused. Slight amplitude modulation is evident in calls with maximum energy being present in the first third of the call's duration. Numerical parameters of 14 analyzed calls are as follows: call duration (= note duration) 74–134 ms (98.3±18.3 ms); dominant frequency 3854–4207 Hz (4069±129 Hz); prevalent bandwidth 2000–5200 Hz. Within call series (containing 7–8 calls; maximum duration of call series 2172 ms), call rate varied from 163–255 calls/minute.

Distribution. The species is known from various sites in northern Madagascar, all at mid- to high-elevation: (1) the type locality Marojejy (Camp Simpona, at mid-elevation), (2) Bemanevika, (3) the southern slope of the Tsaratanana Massif, (4) the western slope of Makira Reserve, and (5) Anjanaharibe-Sud Reserve, based on specimen CRH1693 (KU 347374) included in the FrogCap analysis (Fig. 8). At Makira (west), Bemanevika and Tsaratanana, the species occurs syntopically with *G. razoky* **sp. nov.** (described below). The species is known from elevations between 1093 and 2021 m a.s.l.

Etymology. The name is derived from the Malagasy word razandry meaning smaller (younger) sibling, and makes reference to the fact that this species is the smaller-sized relative of the syntopic larger-sized species of the *G. liber* complex described in the following. The name is used as a noun in apposition to the genus name.

Guibemantis razoky sp. nov.

https://zoobank.org/5121CED1-7B49-455C-94FF-17C77E-A7EDCE

Figs 9, 12, 13

Holotype. ZSM 1746/2010 (field number ZCMV 12515), adult male, collected in Bemanevika 'Camp 1' (Antsirakala; 14.43061°S, 48.60179°E, 1466 m a.s.l.), Sofia Region, northern Madagascar on 27 June 2010 by M. Vences, D. Vieites, R.D. Randrianiaina, F. Ratsoavina, S. Rasamison, A. Rakotoarison, E. Rajeriarison and T. Rajoafiarison.

Paratypes. A total of 27 specimens: ZSM 1744–1745/2010, 1747–1750/2010, 1837/2010, 540–543/2014 (field numbers ZCMV 12513, 12514, 12516, 12523, 12531, 12532, 12539; DRV 6339–6341, 6366), adults and subadults, with same collection data as holotype; ZSM 1751–1753/2010 (field numbers ZCMV 12558, 12574, 12591), two males and one female, collected near Bemanevika River (14.48251°S, 48.62723°E, 1109 m a.s.l.) on 29 June 2010 by M. Vences, D. Vieites, R.D. Randrianiaina, F. Ratsoavina, S. Rasamison, A. Rakotoarison, E. Rajeriarison and T. Rajoafiarison; ZSM 70–73/2016 (field numbers MSZC 0036, 0070, 0144, 0157), four adult males collected from a *Pandanus* swamp at Ampotsidy (14.41694°S, 48.71449°E, 1371 m a.s.l.) on 6 January 2016 by M.D. Scherz, J. Borrell, L. Ball, T. Starnes, E. Razafimandimby, D.H. Nomenjanahary and J. Rabearivony; ZSM 74–75/2016 (field numbers MSZC 204, 240), two adult males collected at Andranonafindra forest (30 km SW of Bealanana on the RN31; 14.73600°S, 48.54831°E, 1180 m a.s.l.) on 14 January 2016, by M.D. Scherz and M. Rakotondratisma; ZSM 877–878/2003 (field numbers FGMV 2002.874, 2002.875), two adult males collected on Montagne d'Ambre (precise coordinates not taken) on 17 February 2003 by F. Glaw, R.D. Randrianiaina and A. Razafimanantsoa;

ZSM 890–892/2003 (field numbers FGMV 2002.898, 2002.899, 2002.900), two males and one female, collected at Montagne d'Ambre, Voie des mille arbres (approximately at coordinates 12.520°S, 49.176°E, 1052 m a.s.l.) on 18 February 2003 by F. Glaw, R.D. Randrianiaina and A. Razafimanantsoa; ZSM 120/2018 (field number MSZC 712), an adult male, collected on Montagne d'Ambre (near Lac Maudit: 12.58528°S, 49.15094°E, 1249 m a.s.l.) on 1 December 2017 by M.D. Scherz, J.H. Razafindraibe, A. Razafimanantsoa, O. Randriamalala, S.M. Rasolonjavato, R.T. Rakotonindrina and A. Rakotoarison; ZSM 119/2018 (field number MSZC 520), an adult male collected on Montagne d'Ambre (12.51994°S, 49.17274°E, 1044 m a.s.l.) on 25 December 2017 by M.D. Scherz, J.H. Razafindraibe, A. Razafimanantsoa, O. Randriamalala, S.M. Rasolonjavato, R.T. Rakotonindrina and A. Rakotoarison.

Diagnosis. This species corresponds to the mitochondrial lineages NOR+NCENTR as defined herein. It is assigned to the subgenus *Pandanusicola* of the genus *Guibemantis* based on presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), moderate to weakly expressed webbing between toes, connected lateral metatarsalia, the presence of both inner and outer metatarsal tubercles, femoral glands in males, absence of nuptial pads, moderately small body size (SVL 26.5–33.9 mm in males and 29.8–32.8 mm in females), and molecular phylogenetic relationships. Within *Pandanusicola*, the new species is distinguished from all species except *G. liber*, *G. razandry*, and *G. tasifotsy* by femoral glands type 1 (vs. type 2) as defined by Glaw et al. (2000), thus possessing many small gland granules in a relatively diffuse field covering most of the thigh ventrally, and by its probable breeding in open swamps (vs. phytotelmic breeding in *Pandanus* leaf axils). It can be distinguished from *G. tasifotsy* by its different brownish color pattern lacking a green dorsal and lateral coloration with series of distinct white blotches along the lower flanks, and its different advertisement call, namely a short click-like note of 20–117 ms duration and 2598–3010 Hz dominant frequency (vs. a longer trill-like note of 147–516 ms duration and higher dominant frequency; Lehtinen et al. 2012). The new species differs from all *G. liber* lineages occurring in the Northern and Southern Central East of Madagascar by its high DNA divergence > 2.4% in the mitochondrial 16S gene, by a larger SVL, and differences in the advertisement call. It differs from *G. razandry* (described above) by larger SVL, different advertisement call, and a molecular 16S divergence >5.5%. It also differs from *G. liber* and *G. razandry* by 3 and 41 diagnostic positions in the analyzed fragment of the cytochrome *b* gene, respectively (see Appendix 2 for a list of diagnostic sites). For a distinction from the third new species described herein, see below.

Description of holotype. Adult male in good state of preservation (Fig. 9). A small piece of muscle tissue from right thigh removed for molecular analysis. Ventral skin cut open and bladder removed for parasite examination. SVL 28.0 mm. For full morphometric measurements see Table 1. Body relatively slender; head slightly longer than wide, wider than body; snout rounded in dorsal, ventral, and lateral views; nostrils much nearer to tip of snout than to eye, slightly protuberant and pointed anterolaterally; canthus rostralis distinct, straight; loreal region straight; tympanum distinct, relatively small, its diameter 70% of eye diameter; distinct supratympanic fold; tongue ovoid, strongly bifid posteriorly; posterior tongue extensions slightly serrated; vomerine teeth as one weakly expressed rounded aggregation posterolateral of each choana; choanae small, rounded. Forelimbs slender; subarticular tubercles distinct and single; central metacarpal tubercle large and rounded, outer metacarpal tubercle smaller and oval; a small but indistinct prepollex (which could also be considered as an inner metacarpal tubercle) at base of first finger. Fingers without webbing; relative finger length I<II<IV<III; finger discs distinctly enlarged; nuptial pads absent. Outer toe and finger discs darker than inner toe and finger discs. Hind limbs long and slender; when adpressed along body, the tibiotarsal articulation reaches beyond the eye; lateral metatarsalia connected by tissue; inner metatarsal tubercle distinct, larger than outer; outer metatarsal tubercle distinct; webbing formula of the foot 1(traces), 2i(traces), 2e(1), 3i(2), 3e(1), 4i(2.75), 4e(3), 5(1.25); relative toe length I<II<III = V<IV. Skin dorsally smooth; ventral skin as far as recognizable smooth on throat, chest, slightly granular on belly. Femoral glands recognizable from external view but not very distinct, also in life (Fig. 12) not very prominent and of same color as surrounding shank, possibly because the specimen was collected outside of the reproductive season. Glands consisting of many small gland granules in a relatively diffuse field covering most of thigh ventrally, thus of type 1 as defined by Glaw et al. (2000).

After eleven years in preservative (70% EtOH; Fig. 9), dorsal background coloration grayish brown with two prominent blackish dorsolateral discontinuous bands consisting of densely arranged blotches and extending posteriorly from eye orbits to hips. Rostral stripe dark brownish. Dorsally, from between orbits a dense field of brown blotches, running over most of dorsum and becoming more scattered on posterior dorsum. Interrupted and indistinct whitish middorsal line. Forelimbs with irregular and partially interrupted dark, brownish bands and spots extending from shoulders to fingers. Outer finger discs darkish brown. Ventrally, throat and forelimbs largely unpigmented (yellowish-brownish in preservative), chest with some fine dark brown dotting, belly more pigmented with dense pattern of brown dots that leave out some larger unpigmented markings, and the hindlimbs dark largely brown. Dorsal surface of thighs and shanks dark reddishbrown with indistinct broad dark blotches and interrupted bands. Like finger discs, some outer toe discs dark reddish brown, of noticeably different color than adjacent tissue.

Based on photographs of the holotype specimen (Fig. 12), body coloration in life was pinkish brown. Dorsally and on both forelimbs and hindlimbs, with a chocolate

brown pattern, consisting of irregular-sized blotches and spots. Also, supratympanic fold and rostral stripe chocolate brown. Dorsally, from an imaginary line between the orbits to the axilla with a dense field of brown blotches. A yellowish interrupted and indistinct middorsal line. Ventrally, background color pinkish-whitish, but chest, throat and the anterior part of belly bright white. Posteriorly and laterally belly semi-transparent. Ventrolaterally, small white spots in anterior part, larger bright yellow spots posteriorly. First finger and toe intensely yellow. Numerous femoral gland granules visible, but not highlighted in color. Iris (whitish in preservative) copper metallic in life.

Variation. Specimens in the type series show differences in color pattern. For instance, in preservative two specimens of the NOR lineage, ZSM 890/2003 and ZSM 877/2003, have a distinct light vertebral stripe while ZSM 878/2003 is dorsally more or less uniformly brownish. In the NCENTR lineage, ZSM 1748/2010 has highly contrasted beige markings on a brown dorsum, including one beige patch anterior to the eyes. While ZSM 1837/2010 is, again, mostly uniform brown dorsally, and ZSM 1747/2010 is dorsally primarily beige, with some dark brown spots and incomplete light vertebral stripe laterally bordered by dark brown. Males collected during the breeding season have a distinct bright white throat, which is not obvious in any of the specimens collected at Bemanevika in June, making it difficult to sex them; however, many of these specimens appear to be males based on gonad examination, suggesting that outside of the breeding season males do not have white throats/vocal sacs. There seems to be no obvious sexual size dimorphism as is typical for the *G. liber* complex. For variation in morphometric features see Table 1.

Natural history. In November to December of 2017 we observed numerous congregations of calling males of *G. razoky* on Montagne d'Ambre. One congregation on the shore of Lac Maudit (ca. 1320 m a.s.l.) consisted of several males and clutches of eggs (Fig. 13F–H), suggesting that the tadpoles of the species may even enter this large lake. A massive congregation, consisting of dozens of specimens and possibly hundreds of clutches, was found in a temporary swamp at ca. 1000 m a.s.l. (Fig. 13A–D). Among this congregation were also *Blommersia wittei* and *G. albomaculatus*. Clutches of eggs were observed to be predated by wasps and ants (Fig. 13E). In Ampotsidy, calling individuals and clutches were found in a swamp with large *Pandanus* plants in December. Outside of the breeding season (in June) we found specimens near Bemanevika River hidden in the leaf axils of *Pandanus* screw pines, syntopic with *Blommersia blommersae*, *Guibemantis* sp. aff. *pulcher*, and *G. razandry*. As with apparently most lineages in the *G. liber* complex, *G. razoky* occurs both in areas of primary rainforest and in highly degraded and fragmented forest patches, e.g., near Bemanevika.

Vocalization. Advertisement calls recorded on 14 March 1994 at Montagne d'Ambre National Park (air temperature 21.2°C) consists of a single, click-like note of rather variable duration containing a low number of well-separated pulses (Fig. 17). Calls (= notes) are usually emitted in short series at rather regular intervals. Numerical parameters of 24 analyzed calls are as follows: call duration (= note duration) 20–117 ms (52.7±29.3 ms); number of pulses per call 2–4 (2.6±0.7); dominant frequency 2598–3010 Hz (2731±120 Hz), with two weaker additional energy peaks at approximately 5400 and 8100 Hz; prevalent bandwidth 2000–8600 Hz. Within call series (containing 3–10 calls; maximum duration of call series 1773 ms), call rate varied from 270–330 calls/minute.

Distribution. Since many *Pandanusicola* species appear to be phenotypically similar and are therefore often taxonomically confused, we restrict our assessment of distribution to populations for which molecular data are available. According to our data, *G. razoky* appears to be a regional endemic of northern Madagascar, and is so far reliably known from six localities (not taking into account the imprecise site "Bealanana region": (1) Bemanevika, the type locality; (2) Ampotsidy; (3) Andranonafrindra forest; (3) Tsaratanana; (4) Makira; (5) Montagne d'Ambre (where a genetically distinct mitochondrial lineage occurs). These localities are from elevations between 1044 and 1466 m a.s.l. Furthermore, genetic samples assigned to this species based on mitochondrial DNA exist from the low-elevation site Ambodiriana (about 50 m a.s.l.) but no voucher specimens from this site are available, and this record thus requires confirmation.

Etymology. The name is derived from the Malagasy word razoky meaning larger (elder) sibling, and refers to the fact that this species is the larger-sized relative of the syntopic *G. razandry*. The name is used as a noun in apposition to the genus name.

Guibemantis fotsitenda sp. nov.

https://zoobank.org/89025EFB-775F-4EF5-8745-9E17DCCC1794

Fig. 9

Holotype. ZSM 292/2005 (field number FGZC 2781), adult male, collected at 'Camp Mantella' in Marojejy National Park (14.43767°S, 49.77555°E, 481 m a.s.l.), Sava Region, northeastern Madagascar on 14 February 2005 by F. Glaw, M. Vences and R.D. Randrianiaina.

Paratypes. Five specimens: ZSM 291/2005 (field number FGZC 2780), adult male with same collection data as holotype; ZSM 289/2005 and 290/2005 (field numbers FGZC 2721–2722), as well as UADBA uncatalogued (FGZC 2723 and FGZC 2724), two unsexed specimens, all collected at a site in between Andrakata and Andapa (geographical coordinates not taken) on 13 February 2005 by F. Glaw, M. Vences and R.D. Randrianiaina.

Diagnosis. This species corresponds to the mitochondrial lineage NE2 as defined herein. It is assigned to the subgenus *Pandanusicola* of the genus *Guibemantis* based on presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), small body size, moderate to weakly expressed webbing between toes, connected lateral metatarsalia, the presence of both inner and outer metatarsal tubercles, femoral glands in males, absence of nuptial pads, small body size (SVL 25.1–26.0 mm in males; female size unknown), and molecular phylogenetic relationships. Within *Pandanusicola*, the new species is distinguished from all species except *G. liber*, *G. razandry*, *G. razoky*, and *G. tasifotsy* by femoral glands type 1 (vs. type 2) as defined by Glaw et al. (2000), thus possessing a large number of small gland granules in a relatively diffuse field covering most of the thigh ventrally, and by its probable breeding in open swamps (vs. phytotelmic breeding in *Pandanus* leaf axils). It can be distinguished from *G. tasifotsy* by its different brownish color pattern lacking a green dorsal and lateral coloration with series of distinct white blotches along the lower flanks. The new species differs from all *G. liber* lineages occurring in the Northern Central East and Southern Central East of Madagascar by its high DNA divergence > 5% in the mitochondrial 16S gene, and probably by a somewhat smaller snoutvent length. *Guibemantis razoky* (see above) has a larger body size (26.5–33.9 mm in males, vs 25.1–26.0 mm in males of *G. fotsitenda*). *Guibemantis razandry* (see above) is the closest relative of *G. fotsitenda* **sp. nov.**, and no obvious morphological differences between these two species are known, despite their clear divergence in mitochondrial and nuclear-encoded DNA in near-sympatry. The new species differs from *G. liber*, *G. razandry*, and *G. razoky* by 23, 23, and 50 diagnostic positions in the analyzed fragment of the cytochrome *b* gene, respectively (see Appendix 2 for a list of diagnostic sites).

Description of holotype. Adult male in good state of preservation (Fig. 9). Pieces of muscle tissue removed from both left and right thigh for molecular analysis. SVL 25.5 mm. For full morphometric measurements see Table 1. Body relatively slender; head slightly longer than wide, wider than body; snout slightly pointed in dorsal and lateral views, rounded in ventral view; nostrils much nearer to tip of snout than to eye and pointed anterolaterally; canthus rostralis relatively distinct, slightly concave; loreal region concave; tympanum distinct, relatively small, its diameter 60% of eye diameter; distinct supratympanic fold; tongue ovoid, distinctly bifid posteriorly; vomerine teeth as one weakly expressed rounded aggregation posterolateral of each choana; choanae small, rounded. Forelimbs slender; subarticular tubercles distinct and single; central metacarpal tubercle large and rounded, outer metacarpal tubercle smaller and oval; a small but indistinct prepollex (which could also be considered as an inner metacarpal tubercle) at base of first finger. Fingers without webbing; relative finger length I<II<IV<III; finger discs distinctly enlarged; nuptial pads absent. Outer toe and finger discs darker than inner toe and finger discs. Hind limbs long and slender; when adpressed along body, tibiotarsal articulation reaches center of eye; lateral metatarsalia connected by tissue; inner metatarsal tubercle distinct, larger than outer; outer metatarsal tubercle distinct; webbing formula of the foot 1(traces), 2i(traces), 2e(1), 3i(2.25), 3e(1.25), 4i(2.5), 4e(2.75), 5(1); relative toe length I<II<V<III<IV. Skin dorsally smooth; ventral skin smooth on throat and chest, slightly granular on belly. Femoral glands not intact due to tissue excision.

After sixteen years in preservative (70% EtOH; Fig. 9), dorsal background coloration light brownish with irregular beige mottling, and several scattered dark brown spots. Area above eyes dark brown, a thin interocular band with a small beige medial interruption. A dark brown rostral stripe is present. Tympanic area dark brown. Several dark brown crossbands present on hindlimbs. Ventral side without dark color elements; belly of a faded beige, throat bright white. Coloration in life not recorded.

Variation. The four available specimens (all males) are morphologically and morphometrically rather similar to each other (Table 1). ZSM 291/2005 has a thin light vertebral stripe while ZSM 289/2005 has a distinct and broad light middorsal band. Femoral glands are well visible in paratype ZSM 290/2005; here, in preservative, they are relatively distinct from external view, consisting of many small gland granules in a diffuse field covering most of the thigh ventrally, thus of type 1 as defined by Glaw et al. (2000).

Natural history. No natural history observations on this species were made, but its habits and habitat are likely similar to those of other species of the *G. liber* complex. It occurs both in intact primary rainforest (Marojejy) and in degraded forest (Andrakata-Andapa). Vocalizations of this species have not been recorded.

Distribution. The species is reliably known from two sites in northern Madagascar: (1) the type locality Marojejy (Camp Mantella, at low elevation), and (2) a site between Andrakata and Andapa also located at rather low elevation. Furthermore, individuals from (3) Ambodivoangy at the north-eastern edge of the Makira Reserve, at ca. 30 m a.s.l., are provisionally assigned to this species based on evidence from nuclear genes, despite their assignment to *G. razandry* based on mitochondrial DNA (see Discussion below). This seems to be a species specialized to habitat at low elevations (known from near sea level to ca. 480 m a.s.l.).

Etymology. The name is derived from the Malagasy words fotsy meaning white, and tenda meaning throat, referring to the white throat (vocal sac) typical for this and other species of the *G. liber* complex. The name is used as a noun in apposition to the genus name.

Discussion

In this study, we have presented evidence that *Guibemantis liber* as previously understood consisted of more than one species-level lineage, and have taken a first step towards taxonomically resolving this species complex. As with other supposedly widespread species of amphibians in Madagascar (e.g., Köhler et al. 2015; Scherz et al. 2019; Rancilhac et al. 2020; Vences et al. 2021, 2022), this required tackling two independent sets of challenges: first, obtaining a dense sampling across the range of the species complex to identify and delimit lineages; and secondly, the nomenclatural hurdle of assigning available names to species.

Since we observed several instances of syntopic occurrence of lineages without genetic admixture in northern Madagascar, and in part with maintenance of morphological differences, we became confident that multiple species were hidden under the name *Guibemantis liber* as previously understood. The concordant differentiation in numerous unlinked genetic markers plus bioacoustic and morphological differentiation confirmed this to be the case. Because all three earlier available names in the complex (*liber*, *albogularis*, *variabilis*) had their type localities in central eastern Madagascar, we were able to scientifically name three northern lineages as new species.

However, resolution of the remaining lineages remains pending and will require a combination of new fieldwork to collect additional genetic, bioacoustic and morphological data, as well as increased scrutiny of the type material of the available names. Specifically, we identify the following research activities needed for a full comprehension of the taxonomy of the *G. liber* complex: (1) targeted collection of tissue samples from additional specimens in the contact zone of lineages NCE1 and NCE2 (e.g., Andasibe) to understand their degree of genetic admixture or lack thereof; (2) recording advertisement calls from further genotyped specimens of lineages NCE1, NCE2 and SCE to verify their presumed bioacoustic differences, as well as first recordings of NCC and SOE whose calls so far remain unknown; (3) obtaining additional morphometric data from genotyped specimens of SCE and SOE, and first morphometric data for SCC; (4) close the sampling gap between SCE/SOE and NCE1/NCE2/NCC lineages, to understand the geographic pattern of hybridization and genetic admixture between these lineages; (5) obtain fresh samples from Itremo, to genetically characterize this population from the type locality of *variabilis*; and (6) obtain fresh samples from Andrangoloaka, the type locality of *liber* and possibly of *albogularis*, to verify our hypothesis that this locality is populated by lineage NCE1. Furthermore, it might be worth examining other morphological characters such as osteology and larval morphology, although Vejarano et al. (2006) found no obvious morphological differences between *G. liber* tadpoles from Ranomafana and Andasibe (corresponding to lineages NCE1 or NCE2 vs. SCE).

While these new field data, in particular new material from Itremo and Andrangoloaka, would help to assign the three available names to lineages, a preferable course of action would be to genetically characterize their name-bearing types, as has been done by a DNA barcode fishing strategy in several other Malagasy anurans (e.g., Rancilhac et al. 2020; Scherz et al. 2020; Vences et al. 2021, 2022).

One intriguing aspect of the molecular phylogenies inferred herein is the apparent paraphyly of the *Guibemantis liber* complex, both in the 16S phylogram (Fig. 1) and the multigene phylogeny (Fig. 7). In both trees, other species of *Guibemantis* of the subgenus *Pandanusicola*, such as *G. annulatus* and *G. wattersoni*, but also *G. pulcher*, *G. albolineatus* and *G. tasifotsy*, were more closely related to either the clade of the two north-eastern lineages of the *G. liber* complex, or to the clade with all other lineages. However, the suggested relationships among these taxa differed between the two trees, and even in the multigene tree, the paraphyly of the *G. liber* complex was not supported by high posterior probabilities (PP = 0.90 for the relevant node). We did not assemble FrogCap data for the relevant species of *Pandanusicola* to submit this issue to a phylogenomic test, but we hypothesize that more comprehensive datasets will provide evidence for the monophyly of the *G. liber* complex. However, taking into account that *G. liber* often uses *Pandanus* leaf axils as shelter and thus occurs in close proximity with other *Guibemantis* species more closely associated with these plants, introgressive hybridization of one of these species into *G. liber* cannot be ruled out; in such a case, the mitochondrial genome (on which our multigene tree mostly relies) would not reflect the species tree, potentially explaining the apparent paraphyly of the *G. liber* complex in our phylogenetic reconstructions.

We hypothesize that introgressive hybridization also explains the discordance of mitochondrial and nuclear signal for the two samples from Ambodivoangy. These samples, collected at a site near sea level, were grouped by multiple nuclear-encoded genes with the low-elevation species *G. fotsitenda*, and due to biogeographic considerations, we consider it likely they belong to this species. However, mitochondrial DNA placed them with *G. razandry*, and we hypothesize that they possess an introgressed mitochondrial genome of that species. More samples from north-eastern Madagascar are needed to define more closely the contact zone between these two species.

According to our data, the *Guibemantis liber* complex represents one additional group of underestimated diversity among Madagascar's herpetofauna. While we found evidence for species-level divergences in this complex based particularly on the syntopic occurrence without admixture of two lineages in northern Madagascar, it is much more difficult to decide in other cases whether identified lineages may represent distinct species, or intraspecific variation that may be best classified at the subspecies level. Future work will likely include population-genomic analysis of hybrid zones (Dufresnes et al. 2021) of such lineages, to understand whether they admix over a wide geographic area or only hybridize along a narrow zone which would be indicative of species-level differ-

entiation. Clearly, for field biologists, it is frustrating to be faced with an increasing number of morphologically cryptic species that are extremely hard to identify without genetic data, but understanding the true diversity of Madagascar's biota – including such cryptic species – is of importance for biogeographic and evolutionary studies, and for conservation assessment. For instance, the *G. liber* complex is one additional group where deeply divergent lineages populate the Southern Central East (SCE/SOE) vs. the Northern Central East (NCE1/NCE2), possibly separated by the Mangoro River Basin. Similarly, the divergence between *G. razok*y (NCENTR/NOR) and the NCE1/NCE2 lineages of *G. liber* mirrors the finding in other groups such as the geckos *Paroedura gracilis* and *Phelsuma guttata*, although in these species the limit between lineages from the North East and Northern Central East is positioned more southwards than in *G. liber* (Mohan et al. 2019).

Guibemantis liber has been considered as a widespread species, occurring in numerous protected areas across Madagascar, and has therefore been assessed as Least Concern in the Red List of the International Union for Conservation of Nature (IUCN SSC Amphibian Specialist Group 2016). The taxonomic revision herein yielded three additional species, and excluded northern Madagascar from the range of *G. liber* sensu lato. Of the three new species, *G. razandry* and *G. razoky* are widespread across northern Madagascar, occur in various protected areas and persist in areas affected by a moderate amount of habitat degradation, suggesting they are not threatened. Also *G. fotsitenda* may tolerate habitat degradation, but it has been found in only a small area of the North East of Madagascar where it is reliably known from only two sites. This species may require more attention in future Red List assessments. For the remaining lineages of the *G. liber* complex, threat status assessments are impeded by the need for in-depth taxonomic revision, but except for the NCC lineage, all of them seem to be widespread and occurring in multiple protected areas, and thus not under immediate threat of extinction.

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Appendix 1

Call descriptions of lineages assigned to *Guibemantis liber*

In the following, we describe calls of different lineages assigned to *G. liber*:

Advertisement calls recorded on 8 February 2000 at Mandraka (probably lineage NCE1; air temperature 18.4°C) consist of a single pulsed note containing a high number of well-separated pulses (Fig. 14). Calls (= notes) are usually emitted in short series at regular intervals. Call energy is distributed in a rather narrow frequency band. Numerical parameters of 14 analyzed calls are as follows: call duration (= note duration) 88–131 ms (112.6±18.0 ms); number of pulses per call 11–22 (16.6±3.9); dominant frequency 2702–3162 Hz (2911±194 Hz); prevalent bandwidth 2000–3500 Hz. Within call series (containing 5 calls; maximum duration of call series 1857 ms), call rate varied from 145–174 calls/minute.

Advertisement calls recorded on 4 February 1994 at Andasibe (probably lineage NCE1; air temperature 24.0°C) are very similar to those from Mandraka, but often lack clearly separated pulses, as these are largely fused. Pulses were countable in only part of the calls analyzed (n = 5). Numerical parameters of 10 analyzed calls are as follows: call duration (= note duration) 75–128 ms (104.7±17.2 ms); number of pulses per call 14–19 (17.0±2.1); dominant frequency 2853–3220 Hz (2873±121 Hz); prevalent bandwidth 1800–3300 Hz. Within the call series, call rate varied from 235–251 calls/minute.

Advertisement calls recorded on 3 March 1996 at An'Ala (probably lineage NCE1; air temperature 22.8°C) generally agree in character with those described from Mandraka and Andasibe but are shorter in duration and contain a lower number of pulses. Numerical parameters of 4 analyzed calls are as follows: call duration (= note duration) 49–75 ms (61.5±11.1 ms); number of pulses per call 7–12 (9.0±2.4); dominant frequency 2691–3044 Hz (2878±147 Hz); prevalent bandwidth 1700–3300 Hz. Within the call series (containing 4 calls), call rate was 156 calls/minute.

Advertisement calls recorded on 12 February 2008 at Ambodisakoa (clade NCE2), near Mahasoa (estimated air temperature around 25°C) consist of a single pulsed note containing a high number of pulses (Fig. 15). Pulses are partly fused, but usually recognizable as countable units. Pulse rate within notes varies from 255–285 pulses/second. Call energy is distributed in a very narrow frequency band. Amplitude modulation is evident with call energy decreasing from approximately the middle of the call towards its end. Calls (= notes) are usually emitted in series at regular intervals and slow succession. Numerical parameters of 13 analyzed calls are as follows: call duration (= note duration) 135–195 ms (162.9±15.3 ms); number of pulses per call 32–48 (35.5±4.6); dominant frequency 3165–3304 Hz (3229±98 Hz); prevalent bandwidth 2300–3600 Hz. Within call series, call rate varied from 28–30 calls/minute.

Advertisement calls recorded on 20 January 2004 near Vohiparara, Ranomafana region (lineage SCE; air temperature 19.5°C) consist of a single short pulsed note containing a low number of pulses, which are largely fused (Fig. 16). Since only poor recordings are available, it is uncertain whether the recorded calls described in the following are indeed typical advertisement calls emitted by highly motivated males. Calls (= notes) are usually emitted in short series at regular intervals. Call energy is distributed in a very narrow frequency band. Numerical parameters of 11 analyzed calls are as follows: call duration (= note duration) 21–35 ms (27.5±4.5 ms); number of pulses per call 4–7 (5.1±1.0); dominant frequency 2919–3090 Hz (2967±71 Hz); prevalent bandwidth 2700–3500 Hz. Within call series, call rate was approximately 280 calls/minute.

For comparison, descriptions of the advertisement calls of *G. razandry* and *G. razoky* (Figs 17–18) are given in accounts of these species.

Appendix 2

Diagnostic positions in the cytochrome *b* gene

The following lists nucleotide positions in the mitochondrial gene (relative to the full cytochrome b sequence of *Mantella baroni*; NC_039758) found to be diagnostic between pairs of species of the *Guibemantis liber* complex in an analysis with DNAdiagnoser.

G. liber vs. *G. fotsitenda*: 500 (R vs. Y), 509 (T vs. C), 530 (D vs. C), 566 (T vs. C), 614 (R vs. C), 645 (T vs. C), 668 (R vs. T), 686 (C vs. T), 698 (T vs. C), 725 (S vs. T), 726 (C vs. T), 767 (C vs. T), 786 (C vs. T), 788 (T vs. A), 821 (C vs. T), 833 (A vs. C), 842 (C vs. T), 869 (R vs. C), 897 (C vs. T), 923 (T vs. C), 953 (C vs. T), 956 (V vs. T), 995 (T vs. C)

G. liber vs. *G. razandry*: 530 (D vs. C), 581 (C vs. T), 614 (R vs. Y), 686 (C vs. T), 698 (T vs. C), 716 (C vs. T), 719 (Y vs. R), 725 (S vs. T), 726 (C vs. T), 788 (T vs. R), 821 (C vs. T), 842 (C vs. T), 857 (M vs. T), 869 (R vs. Y), 896 (T vs. C), 897 (C vs. T), 923 (T vs. C), 953 (C vs. T), 974 (A vs. G), 995 (T vs. C)

G. liber vs. *G. razoky*: 542 (A vs. T), 896 (T vs. C), 986 (T vs. C)

G. fotsitenda vs. *G. razandry*: 500 (Y vs. R), 509 (C vs. T), 557 (G vs. A), 566 (C vs. T), 581 (C vs. T), 645 (C vs. T), 668 (T vs. A), 674 (C vs. T), 677 (T vs. C), 716 (C vs. T), 719 (C vs. R), 731 (T vs. C), 737 (C vs. T), 779 (C vs. T), 836 (T vs. C), 857 (C vs. T), 872 (T vs. C), 896 (T vs. C), 903 (T vs. C), 918 (C vs. T), 956 (T vs. A), 974 (A vs. G), 983 (T vs. C)

G. fotsitenda vs. *G. razoky*: 500 (Y vs. G), 509 (C vs. T), 512 (T vs. A), 530 (C vs. T), 542 (A vs. T), 551 (C vs. T), 555 (T vs. C), 566 (C vs. T), 575 (T vs. C), 578 (C vs. T), 593 (C vs. T), 596 (C vs. T), 614 (C vs. A), 645 (C vs. T), 650 (T vs. C), 656 (T vs. C), 657 (R vs. T), 659 (C vs. T), 668 (T vs. A), 671 (C vs. T), 686 (T vs. C), 698 (C vs. T), 710 (A vs. Y), 725 (T vs. C), 726 (T vs. C), 728 (A vs. G), 731 (T vs. C), 746 (T vs. C), 747 (T vs. C), 755 (T vs. R), 767 (T vs. C), 786 (T vs. C), 788 (A vs. Y), 800 (A vs. G), 824 (T vs. C), 833 (C vs. A), 869 (C vs. G), 875 (T vs. A), 882 (T vs. C), 890 (T vs. C), 896 (T vs. C), 902 (T vs. C), 903 (T vs. C), 905 (A vs. C), 923 (C vs. T), 929 (G vs. T), 956 (T vs. C), 971 (T vs. C), 986 (T vs. C), 1007 (C vs. T)

G. razandry vs. *G. razoky*: 512 (Y vs. A), 530 (C vs. T), 542 (A vs. T), 551 (C vs. T), 555 (T vs. C), 575 (T vs. C), 578 (C vs. T), 581 (T vs. C), 593 (C vs. T), 596 (C vs. T), 614 (Y vs. A), 650 (T vs. C), 657 (A vs. T), 677 (C vs. T), 686 (T vs. C), 698 (C vs. T), 719 (R vs. C), 725 (T vs. C), 726 (T vs. C), 728 (A vs. G), 737 (T vs. C), 746 (T vs. C), 747 (T vs. C), 755 (T vs. R), 779 (T vs. C), 788 (R vs. Y), 824 (T vs. C), 836 (C vs. T), 857 (T vs. C), 869 (Y vs. G), 872 (C vs. T), 875 (Y vs. A), 882 (T vs. C), 902 (T vs. C), 905 (A vs. C), 923 (C vs. T), 929 (R vs. T), 956 (A vs. C), 971 (T vs. C), 974 (G vs. A), 986 (T vs. C)

Supplementary Material 1

List of DNA sequences with metadata

Authors: Koppetsch T, Pabijan M, Hutter CR, Köhler J, Gehring P-S, Rakotoarison A, Ratsoavina FM, Scherz MD, Vieites DR, Glaw F, Vences M (2023)

Data type: .xlsx

Explanation note: List (tab-delimited text) with all Sanger sequences used in this study, associated metadata voucher, locality) and Genbank accession numbers.

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Link: https://doi.org/10.3897/vz.73.e94063.suppl1

Supplementary Material 2

Tables S1–S4, Figs S1–S4

Authors: Koppetsch T, Pabijan M, Hutter CR, Köhler J, Gehring P-S, Rakotoarison A, Ratsoavina FM, Scherz MD, Vieites DR, Glaw F, Vences M (2023)

Data type: .docx

Explanation note: **Table S1.** Primers and thermal cycling profiles used for the amplification of DNA fragments used in this study (see Materials and Methods). Fragment length refers to the alignment length in the concatenated supermatrix; nuclear-encoded genes have been further trimmed for calculation of haplotype networks. Thermal cycling schemes start with temperature (in °C) of each step, followed by the time in seconds between parentheses; cycling repetitions are indicated within brackets. — **Table S2.** Substitution models and partitions applied for the multigene BI phylogenetic reconstruction. — **Table S3.** Minimum and maximum pairwise uncorrected distance for a fragment of the mitochondrial cytochrome *b* gene among the nine main mitochondrial lineages of the *G. liber* complex. — **Table S4.** Minimum and maximum pairwise uncorrected distance for a fragment of the 5'-end of the mitochondrial 16S rRNA gene among the nine main mitochondrial lineages of the *G. liber* complex. — **Figure S1.** Maximum likelihood trees calculated from DNA sequences of fragments of the mitochondrial gene for cytochrome *b*, and from the phased allele sequences for four nuclear-encoded genes, for samples from An'Ala where mitochondrial haplotypes corresponding to the genetic lineages NCE1 and NCE2 occur in syntopy (as obvious from the cytochrome *b* tree). The four nuclear genes do not show any evidence of concordant differentiation of these two lineages at this site. — **Figure S2.** Maximum likelihood trees calculated from DNA sequences of fragments of three nuclear-encoded genes, for lineages NE1 (red) and NE2 (magenta; within box delimited by dotted line). The trees illustrates that samples from Ambodivoangy (highlighted yellow) are assigned to NE2 by the nuclear-encoded markers, suggesting they probably belong to this lineage (= *G. fotsitenda*), in agreement with their occurrence at low elevation, but possess an introgressed mitochondrial genome from NE1 and therefore cluster within NE1 in the mitochondrial tree (Fig. 1). — **Figure S3.** Species tree obtained with ASTRAL from gene trees of 12,951 nuclear-encoded markers. Pie charts represent the quartet score frequency i.e., the proportion of gene trees that support the different topologies. The biggest slice is the current topology and indicates how many gene trees support that topology compared to the others. — **Figure S4.** Dorsal and ventral views of the designated lectotype of *Gephyromantis variabilis* Millot and Guibe, 1951 (MNHN 1953.117) from Itremo.

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