An endless harvest: integrative revision
of the *Gephyromantis boulengeri* and *G. blanci* complexes
reveals six new species of mantellid frogs from Madagascar

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Abstract. The Malagasy genus *Gephyromantis* contains 51 species of primarily terrestrial or scansorial frogs. Although many species are morphologically weakly divergent from each other, the combination of molecular and bioacoustic evidence has led to a continuous flow of species discoveries in the last years. Previous works have notably shown the existence of numerous additional deep mitochondrial lineages of uncertain status in the nominal subgenus *Gephyromantis*, some of these considered as confirmed or unconfirmed candidate species, some as deep conspecific lineages. Here we use DNA sequences of one mitochondrial and one nuclear marker, as well as morphological and bioacoustic data, to conduct an integrative revision of the subgenus *Gephyromantis*. The analyses reveal at least 12 distinct and independent evolutionary lineages belonging to the *G. blanci* and *G. boulengeri* species complexes. Evidence for the species status of these lineages included multiple cases of syntopic occurrence without genetic admixture, as well as differences in advertisement calls or morphological differentiation without intermediate forms, suggesting reproductive isolation. We discuss the relevance of these different lines of evidence and describe six new species of *Gephyromantis*.

Key words. Amphibia, Anura, *Gephyromantis mitsinjo* sp. n., *G. kremenae* sp. n., *G. sergei* sp. n., *G. mafifeo* sp. n., *G. feomborona* sp. n., *G. cornucopia* sp. n., bioacoustics, taxonomy, phylogeny.

Introduction

Madagascar's amphibian fauna is currently composed of 398 described species of frogs (AmphibiaWeb 2022), belonging to five clades that independently diversified on the island (Crottini et al. 2012). The high species diversity of the Malagasy anuran fauna is paralleled by a high ecomorphological and reproductive diversity (Blommers-Schlösser 1979a, b, Glaw & Vences 2007), and by a remarkable density of species and individuals in some ecosystems. Two regions in eastern Madagascar, the Southern Central East (Ranomafana) and Northern Central East (Andasibe), harbor high levels of anuran diversity, with over 100 frog species found in each region within a rather limited area (Vieites et al. 2009). Tadpoles also occur in high densities in mid-elevational headwater streams, possibly favored by the absence of fishes (Strauss et al. 2013). Furthermore, it is remarkable that in Madagascar's rainforests, frogs often are also acoustically dominant (Glaw & Vences 2007). This is particularly obvious at night, during the rainy season, but sometimes also during the day when numerous frogs compete with birds and insects over the acoustic niche (Vences et al. 2006, Glaw & Vences 2007). Central elements of the diurnal acoustic stage are the conspicuous and loud calls of species of the subgenus *Gephyromantis* (*Gephyromantis*). These small frogs occur within the primary rainforest, at rainforest edges, and sometimes in secondary vegetation in the vicinity of rainforest fragments (Blommers-Schlösser 1979a, Glaw & Vences 2007, Wollenberg et al. 2012). The nidicolous reproduction of these frogs, in which non-feeding tadpoles develop in jelly nests (Randrianiaina et al. 2011), allows them to colonise suitable habitat even in the absence of water bodies. This behaviour may explain why their calls can often be heard across the forest, typically from dense, understory vegetation patches.

Gephyromantis is a genus of currently 51 species (AmphibiaWeb 2022) of primarily terrestrial or scansorial frogs. Most species have inconspicuous coloration and secretive habits, except for their often loud and characteristic advertisement calls (Glaw & Vences 2007). Many species within this group exhibit similar morphological characteristics, but the use of molecular and bioacoustic evidence has led to a constant flow of species discoveries in the last years (e.g., Vences & De la Riva 2007, Vieites et al. 2009, 2012, Glaw & Vences 2011, Glaw et al. 2011, Crottini et al. 2011, Wollenberg et al. 2012, Scherz et al. 2017a, b, 2018a, b, Vences et al. 2017, 2021a, 2022, Cocca et al. 2020). *Gephyromantis* is divided into six subgenera (Glaw & Vences 2006, Kaffenberger et al. 2012, Vences et al. 2017) which differ in their habits and reproductive mode: some subgenera are nidicolous with endotrophic tadpoles (*Duboimantis, Gephyromantis, Laurentomantis*), some have generalised exotrophic tadpoles (*Asperomantis*) or specialised carnivorous tadpoles (*Phylacomantis*), or unknown larval stages (*Vatomantis*) (Randrianiaina et al. 2007, 2011, Reeve et al. 2011). Biogeographically, several of the subgenera (e.g., *Duboimantis, Vatomantis*, probably also *Asperomantis* and *Laurentomatis*) have their center of species richness and origin in northern Madagascar (defined as the area north of a diagonal spanning from 15.5° S on the east coast to ca. 15.0° S on the west coast; Brown et al. 2016), while in others, patterns of richness or endemism are less obvious – particularly in the nominal subgenus *Gephyromantis*, with species known from the South East, Southern Central East and Northern Central East of Madagascar (regions after Boumans et al. 2007), but apparently absent from most of northern Madagascar. To fully understand the biogeography and evolutionary history of these frogs, it is necessary to complete their species inventory and assess their distribution ranges.

The subgenus *Gephyromantis* currently contains 10 species: *G. blanci, G. boulengeri, G. decaryi, G. enki, G. hintelmannae, G. leucocephalus, G. mafy, G. runewsweeki, G. thelenae, G. verrucosus*. However, available molecular data (e.g., Vieites et al. 2009, 2012, Wollenberg et al. 2012, Perl et al. 2014) indicate the existence of numerous additional deep mitochondrial lineages of uncertain status in the *Gephyromantis* (*Gephyromantis*) clade, some of these considered as confirmed or unconfirmed candidate species, some as deep conspecific lineages. Past research has focused on a complex comprising *G. decaryi* and allied species (Wollenberg et al. 2012), the *G. eiselti/thelenae/mafy* complex (Wollenberg & Harvey 2010, Vieites et al. 2012), and *G. enki* (Wollenberg Valero 2015). In contrast, taxonomic confusion exists regarding two other species, *G. blanci* and *G. boulengeri*. The first of these species, *Gephyromantis blanci,* was originally described from the Andringitra Massif (Guibé 1973). Specimens and DNA sequences from Ranomafana National Park have been assigned to this species (Vieites et al. 2009), and putatively related (or morphologically similar) candidate species reported under the names *G.* sp. aff. *blanci* "Andohahela" (Glaw & Vences 2007; named *G.* sp. 5 by Vieites et al. 2009 and Kaffenberger et al. 2012; and *G.* sp. Ca5 by Perl et al. 2014) and *G.* sp. aff. *blanci* "Farafangana" (named *G.* sp. 6 by Vieites et al. 2009, Wollenberg et al. 2012, and Kaffenberger et al. 2012; and *G.* sp. Ca6 by Perl et al. 2014). More recently (Kaffenberger et al. 2012, Perl et al. 2014, Belluardo et al. 2021), molecular data of *G. blanci* from the type locality Andringitra have become available and turned out to be highly different from those of the other populations; a detailed analysis has been however missing until now. This is also the case for *G. boulengeri* which was originally described from the locality Folohy close to Toamasina (Methuen 1920), whose precise location is however uncertain (discussed in Vences et al. 2022). Vieites et al. (2009) report three deep conspecific lineages assigned to *G. boulengeri* based on similarities of advertisement call structure, but their morphology, bioacoustics, and molecular relationships have not been studied in depth.

In this study, we undertake a further step towards a complete taxonomic revision of the subgenus *Gephyromantis* within the genus *Gephyromantis*. We complement available DNA sequences for one mitochondrial and one

nuclear gene with 221 new sequences and integrate these molecular data with novel bioacoustic and morphological comparisons. Our data suggest a status of distinct species for four previously identified and two newly discovered lineages, in some cases corroborated by sympatric occurrence without admixture.

Materials and methods
Sampling data

This study is based on samples and voucher specimens collected during expeditions to Madagascar between 2000–2018. Upon collection, specimens were anesthetised by immersion in MS222 or chlorobutanol solution, and subsequently euthanised by an overdose of the same substances (following HACC 2004). We removed tissue samples for molecular analysis and stored them separately in vials with pure ethanol. Voucher specimens were then fixed in 95% ethanol, preserved in 70% ethanol, and deposited at the Zoologische Staatssammlung München (ZSM) and the Université d'Antananarivo, Département de Biologie Animale (UADBA). Additionally, type material was studied from the Ditsong National Museum of Natural History (previously Transvaal Museum) in Pretoria (TM), the Museo Regionale di Scienze Naturali in Turin (MRSN) and the Muséum national d'Histoire Naturelle, Paris (MNHN). ACZC and ACP refer to field numbers and DNA extraction numbers of A. Crottini, FAZC to field numbers of F. Andreone, FGZC, FGMV and ZCMV to field numbers of F. Glaw and M. Vences, JCR to field numbers of J. C. Riemann, and PSG to field numbers of P.-S. Gehring. Geographic regions within Madagascar are given according to Boumans et al. (2007).

Morphological data and analyses

The following morphometric measurements were taken by MV with an accuracy of 0.1 mm using a manual caliper: snout–vent length (SVL); maximum head width (HW); head length from the tip of snout to the posterior edge of snout opening (HL); horizontal tympanum diameter (TD); horizontal eye diameter (ED); distance between the 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 the tip of the longest finger with the limb outstretched (FORL); hand length, to the tip of the longest finger (HAL); hind limb length, from the cloaca to the tip of the longest toe with the limb outstretched (HIL); foot length (FOL); foot length including tarsus (FOTL); and tibia length (TIBL). The webbing formula is given according to Blommers-Schlösser (1979a) to ensure comparability with previous species descriptions of Malagasy frogs.

Exploratory principal component analyses (PCA) were performed with ClustVis (Metsalu & Vilo 2015; online application available at https://biit.cs.ut.ee/clustvis/). Sample sizes were too small to apply allometric growth curves for full allometry corrections, therefore, to remove the main impact of body size on metric measurements, all measurements (except SVL) were size-corrected by dividing them by SVL. This procedure also has the advantage of providing simple ratios that can be easily calculated from raw measurements, even in the field, and used for diagnosis if consistent interspecific differences are detected. Data were normalised prior to the PCA. Exploratory analyses (not shown) detected no obvious sexual dimorphism in most characters and we therefore merged data from males and females for PCA and single-variable comparisons, to detect differences that can be used for diagnosis even for specimens of unknown sex. The final dataset represents a full matrix (specimens with missing data were excluded) of 81 adult specimens from both sexes and 14 quantitative characters.

Bioacoustic data and analyses

Vocalisations were recorded 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 a digital recorder with built-in microphones (Edirol R-09; saved as uncompressed files). For most often, air temperature was measured using a digital thermometer. Recordings were sampled or re-sampled at 22.05 kHz and 32-bit resolution and computer-analysed using the software Cool Edit Pro 2.0. Frequency information was obtained through Fast Fourier Transformation (FFT; width 1024 points) at Hanning window function. Spectrograms were obtained at Blackman window function with 256 bands resolution. Because of partly poor recording quality, sensitive filtering was applied in several cases to remove background sounds, with filtering exclusively applied to frequencies outside the prevalent bandwidths of calls. Temporal measurements are given as a range with mean ± standard deviation in parentheses. Methods used in analyses are those recommended by Köhler et al. (2017), with bioacoustic descriptions following the call-centered terminological scheme. Comparative call data were partly taken from previously published call descriptions (Glaw & Vences 2000, 2002, Vences & De la Riva 2007, Vieites et al. 2012). Soundfiles have been deposited in the Zenodo repositoriy under DOI 10.5281/zenodo.7561864.

Molecular data and analyses

We assembled two molecular data sets: Firstly, we DNA barcoded all available samples using a 3' fragment of the mitochondrial 16S rRNA gene which has previously been used as a standard marker for Madagascar's frogs (Vieites et al. 2009). DNA was extracted using a regular salt extraction protocol (Bruford et al. 1992), and the 16S fragment amplified using the primer pair: 16SAr-L (5'–CGC-CTGTTTATCAAAAACAT–3') and 16SBr-H (5'–CCG-

GTCTGAACTCAGATCACGT–3’) of Palumbi et al. (1991), or with the modified primers 16SFrogL1/16SFrogH1 (5’– CATAATCACTTGTTCTTTAAA–3’; 5’–GATC-CAA CATCGAGGTCG–3’), and the following PCR protocol: initial denaturation at 94°C (90 s), followed by 36–40 cycles of denaturation at 94°C (45 s), primer annealing at 50–53°C (45 s) and elongation at 72°C (90 s), followed by a final extension step at 72°C (5 min). Secondly, as a means to assess concordance between the variation in mitochondrial and nuclear-encoded genes, we amplified a fragment of the recombination-activating gene 1 (RAG1) with the primers Gephlut-RAG1-F1 (5’–ATGGAGAGC-CAACCCCTATC–3’) and Gephlut-RAG1-R1 (5’–KCCA-GACTCGTTTCCTTCRC–3’) of Vences et al. (2021a) with PCR protocol 94(120), [94(20), 53(50), 72(180) x 35], 72(600), and using the newly developed Rag1MantiSeq1 (5’–GCAAAGCCvTTTATTGAAACC–3’) as sequencing primer.

We purified PCR products with Exonuclease I and Shrimp Alkaline Phosphatase digestion. Sequencing was performed on automated DNA sequencers of LGC Genomics (Berlin, Germany) and Macrogen Inc (Amsterdam, The Netherlands; Madrid, Spain). We quality-checked chromatograms and corrected or trimmed sequences, where necessary, with CodonCode Aligner 3.7.1 (Codon Code Corporation, Dedham, MA, USA) and submitted 221 newly determined sequences to GenBank (accession numbers OQ190241 to OQ190311, and OQ198836 to OQ198962; Supplementary document 1).

Sequences were aligned using MAFFT 7, using the FFT-NS-i method and default parameters (online application available at https://mafft.cbrc.jp/alignment/server/, Kuraku et al. 2013, Katoh et al. 2019). Uncorrected pairwise distances between 16S sequences were calculated using the program TaxI2, implemented in iTaxoTools (a software toolkit dedicated to taxonomic research, available at http://itaxotools.org/; Vences et al. 2021b). Phylogenetic analyses of the 16S data were carried out using IQtree, with automatic detection of best-fit substitution model (TIM2+F+I+G4 in the present case) and 5.000 ultrafast bootstrap iterations (Minh et al. 2013, Nguyen et al. 2015; online application available at http://iqtree.cibiv.univie.ac.at/).

ASAP (Puillandre et al. 2021) was used to explore the structure of the 16S dataset and identify the main significant mitochondrial lineages. This delimitation tool infers species partitions from single locus sequence alignment, based on a hierarchical clustering algorithm that only uses pairwise genetic distances (online application available at https://bioinfo.mnhn.fr/abi/public/asap/; Puillandre et al. 2021). We ran ASAP with default parameters, i.e., with a Jukes-Cantor substitution model and a split probability < 0.01. The different alternative partitions obtained were
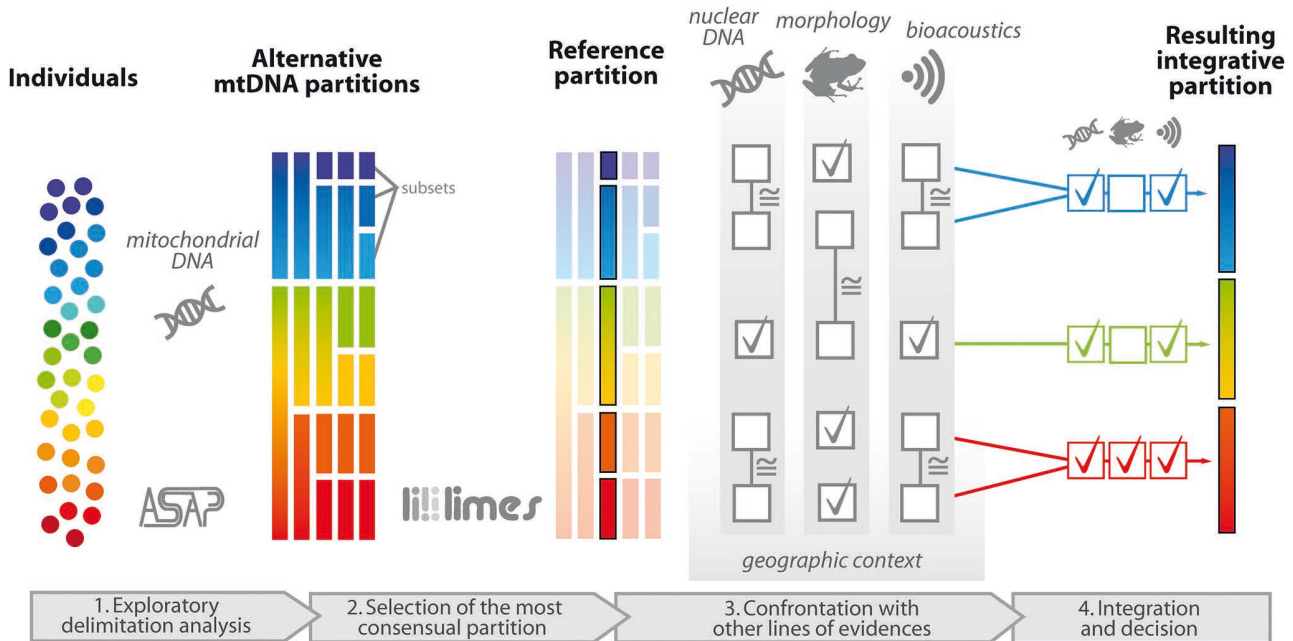

Figure 1. Graphic scheme showing the integrative species delimitation process. A first automated delimitation analysis is performed with ASAP from the mitochondrial DNA dataset (16S). Among the best resulting partitioning schemes, the most consensual one with respect to all is selected using LIMES. This partition is then used as a reference framework to test the different species barriers hypotheses by using a diversified set of independent lines of evidence (i.e., absence of haplotype sharing within the nuclear dataset, significant morphological or bioacoustic differentiation) while interpreting them integratively and taking into account their respective biogeographical contexts (e.g. sympatric co-occurrences). Based on these case-by-case comparative assessments, closely related subsets with insufficient support for distinctiveness are grouped, forming thus a reduced new partition that can be assessed in turn. If necessary, this process is iterated until a new partition is obtained in which the distinctiveness of each subset is sufficiently supported to consider them all as distinct species.

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exported in .spart exchange format (multiple species partitions file; Miralles et al. 2022) and statistically compared, based on the mCtax (mean index of taxonomic congruence; Miralles & Vences 2013) calculated by Limes 2.0 (Ducasse et al. 2020), a species partition comparison tool implemented in iTaxoTools (Vences et al. 2021b). It should be mentioned that the term "partition" here follows the set theory concept: the organisation of a set of elements into mutually-exclusive and jointly-comprehensive subsets, not including the empty subset. In a species delimitation application, the elements are individual samples or specimens, and a specific species delimitation hypothesis corresponds to a particular assignment (i.e. a partition) of these individuals to subsets, where each subset corresponds to a distinct inferred species. Categories resulting from such preliminary SD analysis are usually referred to by various terms, such as primary species hypothesis, operational taxonomic unit (OTUs), barcode index number (BINs; Ratnasingham & Hebert 2013), or even cluster (without any particular status), but all of them match the aforementioned definition of a subset (Miralles et al. 2022).

The RAG1 sequences were analysed separately from the 16S sequences, with the goal of assessing concordance in the differentiation of a nuclear-encoded and a mitochondrial gene. We graphically visualised relationships among alleles (haplotypes) of RAG1 using a haplotype network approach. For this, we first inferred haplotypes of the nuclear genes using the PHASE algorithm (Stephens et al. 2001) implemented in DnaSP (Version 5.10.3; Librado & Rozas 2009). We then reconstructed a Maximum Likelihood tree under the Jukes-Cantor substitution model from these phased sequences in MEGA X (Kumar et al. 2018), and used this tree along with the respective alignment 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). The resulting network has been colourised using a vector graphics editor and connections between distinct haplotypes found co-occurring in heterozygous individuals have been added manually.

Integrative species delimitation

For species delimitation, we follow the general lineage concept (de Queiroz 1998, 2007) but combine it 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 & Wollenberg 1997), we use genealogical concordance (Avise & Ball 1990) between mitochondrial and nuclear loci, especially in populations occurring in sympatry or close geographical proximity as an indicator for restricted gene flow. This is then used to assign species status to a lineage, along with concordance between genetic, morphological and bioacoustic evidence (Padial et al. 2010) (Fig. 1).

Species complex terminology

To ease both reporting and discussing the differentiation among the lineages identified, and based on preliminary morphological, biogeographic and genetic comparisons, we furthermore define the following species complexes in the subgenus *Gephyromantis*: (1) The *G. boulengeri* complex contains comparatively (for the subgenus) large-sized species occurring in low- and mid-elevations, characterised by rather coarsely and irregularly tubercular dorsal skin, absence of dorsolateral ridges, and frenal stripe usually with quite distinct dark patches interrupting the light background colour. (2) The *G. decaryi* complex (also including *G. leucocephalus*), is a morphologically variable monophyletic group occurring primarily in lowlands of the South East and Southern Central East. (3) The *G. eiselti* complex, containing a clade of three small-sized species from mid-elevations in the Northern Central East, with a rather smooth dorsal skin without, or with poorly expressed, dorsolateral ridges, rather continuous white colour on frenal stripe, often with some yellowish colour both dorsally and ventrally and sometimes reddish colour ventrally on limbs. And (4) the *G. blanci* complex, a paraphyletic cluster containing highland species from the South East and Southern Central East, with rather smooth skin, dorsolateral ridges usually present, light frenal stripe often rather continuous, with or without yellow elements in dorsal or ventral colour.

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: lsid:zoobank.org:pub:E5FCFC4E-087D-4F43-BFB1-E58614ED7572. The electronic edition of this work was published in a journal with an ISSN, has been archived and is available from the following digital repositories: www.zenodo.org and www.salamandra-journal.com.

Results
Preliminary partitioning and taxonomic reference framework

Mitochondrial sequence data were available for 244 samples for a fragment of the 16S rRNA gene (total alignment length 509 bp). Confirming previous knowledge, these contained numerous deep lineages of uncertain taxonomic status. To describe this variation and establish a consistent comparative framework suitable for our integrative dataset, we submitted the 16S alignment to a lineage delimitation analysis with ASAP. Then the most consensual par-

tition in regard to the others was selected among the ten best partitions inferred (i.e. the one with the highest mCtax score, among a set of 10 partitions composed of 12 to 56 subsets each). We considered this partition with 19 subsets (LIMES mCtax = 0.63, ASAP threshold distance: 0.028456, ranked 4/10, 5/10 and 9/10 by ASAP, according to W val-

ues (4.85^{e-05}), ASAP score (6.50) and p-values (9.46^{e-01}), respectively) to represent the best compromise in terms of representation of the mitochondrial data-set structure: it is neither too highly split (to reduce as much as possible the intraspecific structural complexity of the dataset) nor too clustered (to preserve a rather fine degree of resolution
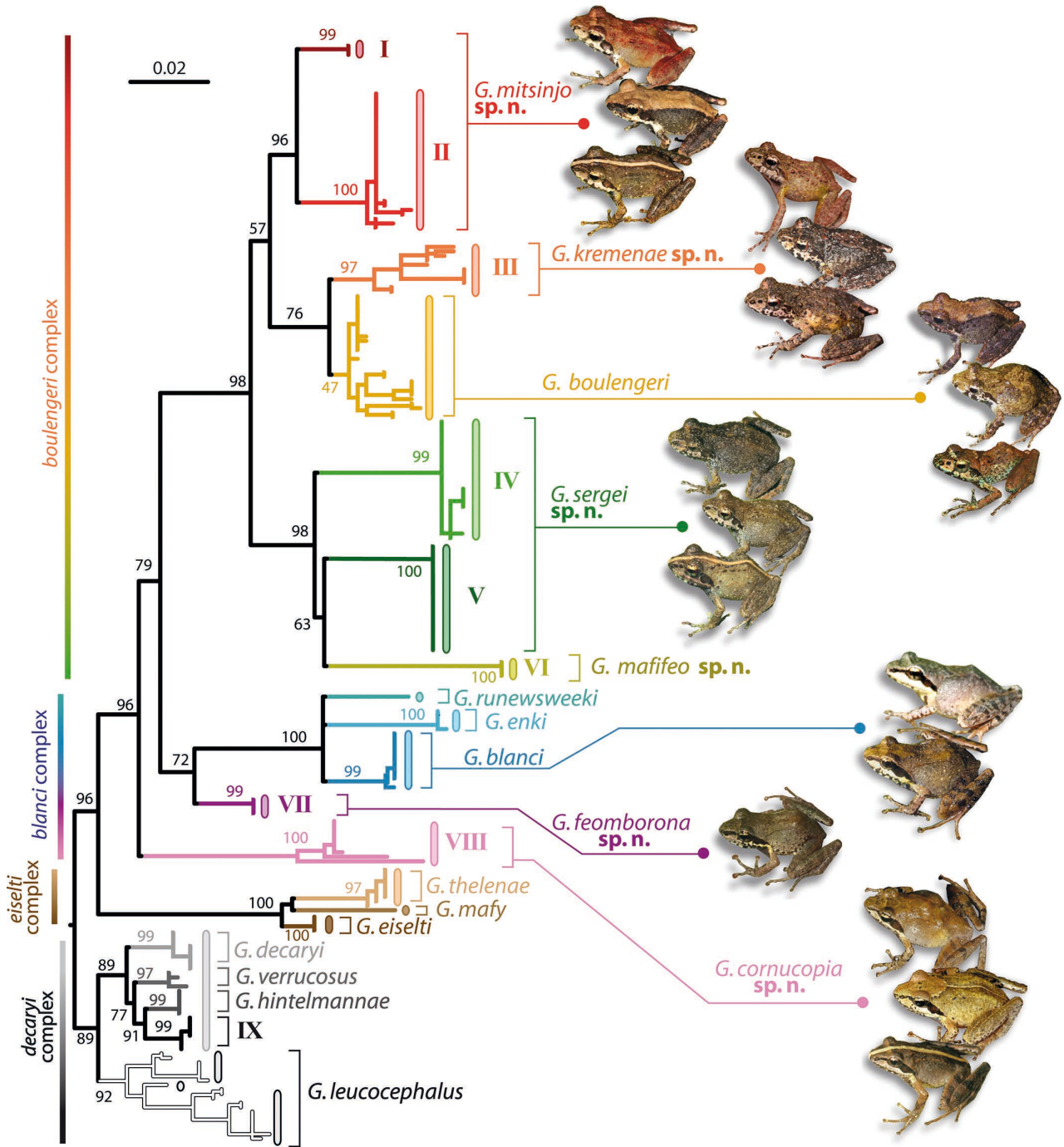

Figure 2. Overview of the best Maximum Likelihood phylogenetic tree based on the 16S dataset (see Fig. 4 for details). Node support (bootstrap scores) is indicated in percentages. Coloured vertical bars represent the 19-subsets partition inferred with ASAP (i.e., the most consensual among the 10 best partitions selected by ASAP).

within the intraspecific genetic structure). This partition was then modified to design a new "operational" partition (with 20 subsets) that will serve as a reference framework across the present study (Fig. 2, see also Fig. 1 and Fig. 3 for the geographic distribution of each subset). For the scope of our study, this operational partition does not differ from the one inferred by ASAP, as the changes concern only subsets within the *G. decaryi* species complex which we will not further consider here. For the sake of clarity, it was more convenient to refer to these subsets by using
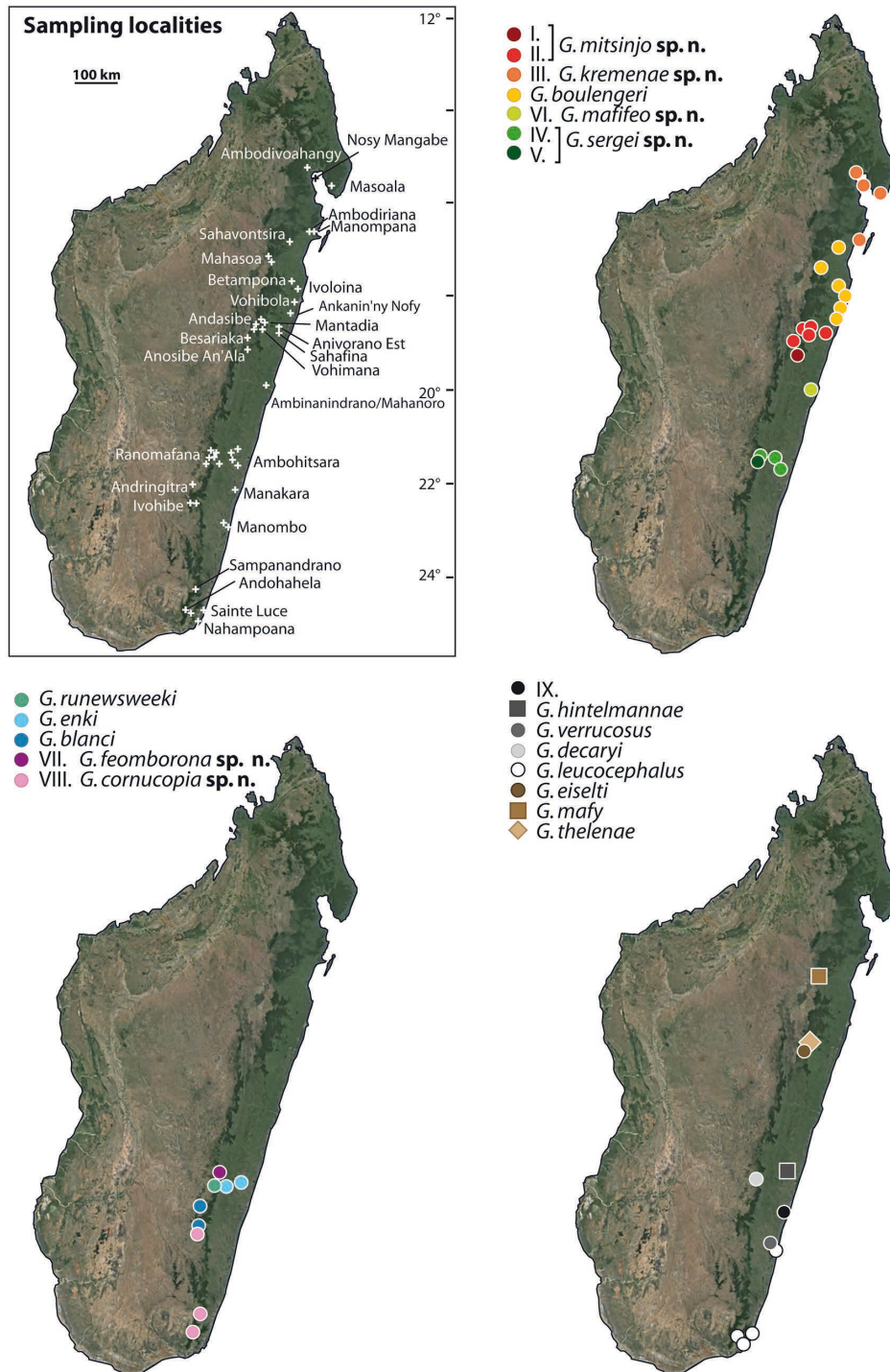

Figure 3. Maps of Madagascar showing the sampling localities and the distribution of the main mtDNA lineages identified (each dot can represent several nearby sites).

the currently accepted binomial species classification, thus reducing discursive complexity to the bare necessities. The operational 20-subset partition will in the following be used as a frame of reference, allowing us (1) to consistently refer to individuals based on their mitochondrial assignment and (2) to graphically represent various results by using a unique colour chart and a common terminology. The 20-subsets are as follows: eleven subsets (equaling mitochondrial lineages) based on the specific epithets of the corresponding species (therefore partly anticipating results), i.e. subsets "boulengeri", "runewsweeki", "enki", "blanci", "thelenae", "mafy", "eiselti", "decaryi", "verrucosus" and "leucocephalus", plus nine subsets (lineages) numbered I to IX. With reference to the previous numbering of candidate species, "boulengeri" corresponds here to the lineage previously named *G.* sp. 25 or *G.* sp. Ca25; subset II corresponds to *G.* sp. 24/Ca24; subset VII corresponds to

G. sp. 4/Ca4; and subset VIII corresponds to *G.* sp. 5/Ca5 (cf. Vieites et al. 2009, Gehring et al. 2010, Kaffenberger et al. 2012, Perl et al. 2014).

16S phylogenetic tree

The tree obtained by Maximum Likelihood analysis of the 16S dataset (Fig. 2, plus details in Fig. 4) was fully resolved, although several deep nodes were too poorly supported to reliably infer interspecific relationships. Nevertheless, several clades such as the *G. decaryi* and *G. eiselti* species complexes were recovered, in agreement with previously published multi-gene phylogenies (e.g., Kaffenberger et al. 2012). Most importantly considering the aim of the present study, almost all of the operational subsets received strong support (bootstrap scores >95%, most often 99 or 100%).
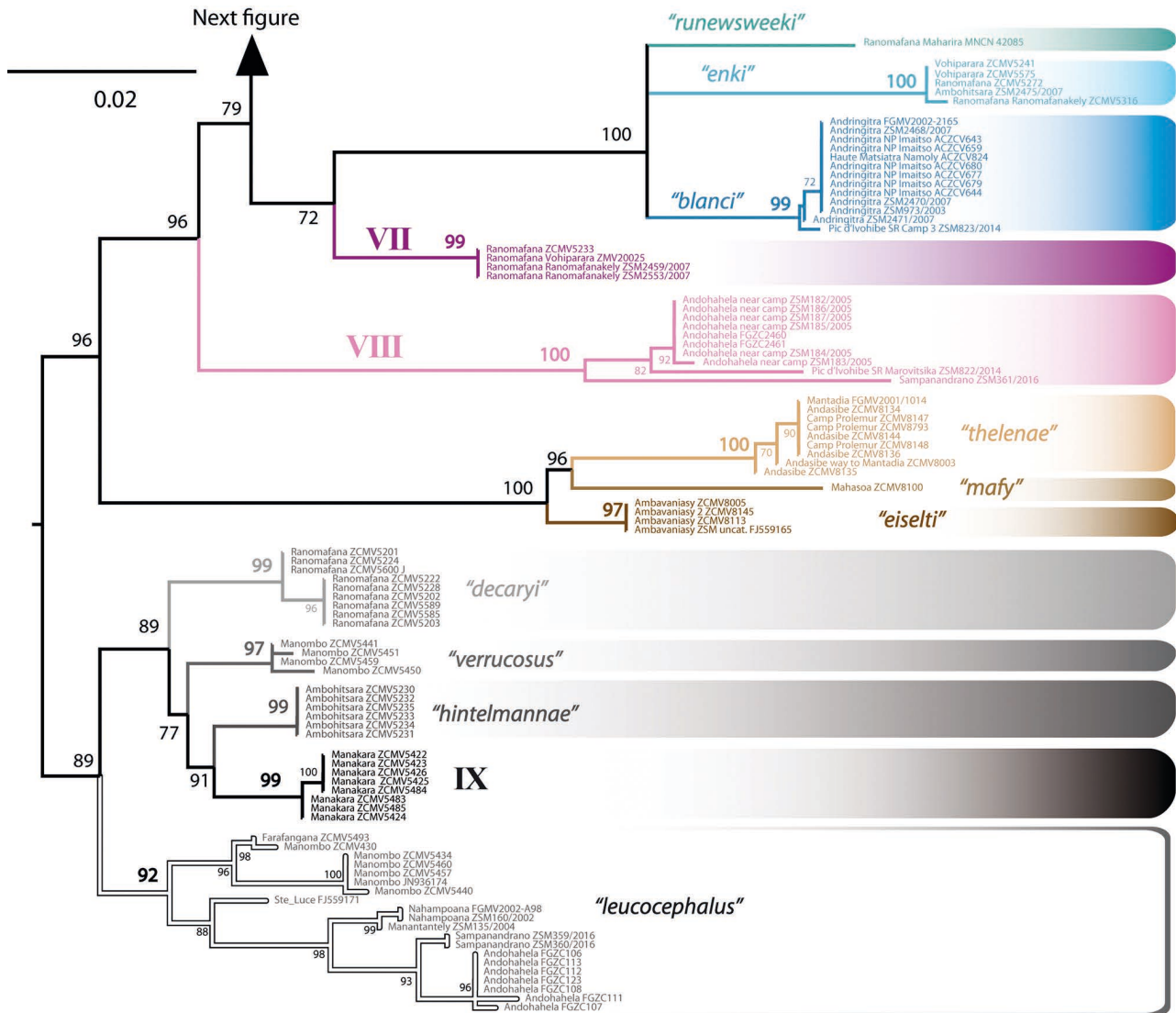

Figure 4. Maximum Likelihood phylogenetic tree of *Gephyromantis* (subgenus *Gephyromantis*) based on the 16S dataset (lower half of the tree).

Figure 4, continued. Maximum Likelihood phylogenetic tree of *Gephyromantis* (subgenus *Gephyromantis*) based on the 16S dataset (upper half of the tree).

Only a single clade, corresponding to the subset "boulengeri", is not supported (47%).

While verifying the locality information of previously published sequences, we noted the need to correct two minor errors, leading to a more consistent picture of the geographic distribution of lineages. First, the DNA sequence with GenBank number AY848329 from a specimen from Moramena likely originates from the Moramena corridor near the Zahamena reserve (the location name Moramena deriving from Moramanga-Zahamena), rather than from a site in Masoala as suggested by the locality information in the supplementary tables of Vieites et al. (2009). This sequence clusters with *G. boulengeri* but due to the remaining uncertainty is not included in our tree and the locality not included in our map. Second, the lineages "Ca24" and *G. boulengeri* (as "Ca25" in Vieites et al. 2009) do not occur in syntopy in Sahafina as suggested by Gehring et al. (2010). Instead, at this site, only sequences of lineage "Ca24" occur, and the specimen ZCMV 8961 (purportedly from Sahafina and belonging to "Ca25") originates from Ambodiriana, as correctly noted in the respective GenBank record (HM631896), and is to be assigned to the subset III of the present study. Due to its short length, we also did not include this sequence in our tree.

Nuclear DNA network (RAG1)

The RAG1 network (Fig. 5) is composed of 41 distinct haplotypes, and presents an overall structuring similar to the mtDNA tree: The specimens of each operational subset (i.e., the 15 for which RAG1 sequences are available) present most often closely related haplotypes in the RAG1 network. Within each of these mitochondrial subsets, RAG1 haplotypes differ most often by only one or two mutational steps, and less frequently by up to a maximum of five. It is worth noting that within these subsets, the most divergent haplotypes are frequently found co-occurring in heterozygous specimens, therefore suggesting that despite significant numbers of mutational steps, they belong in all likelihood to the same current gene pools (e.g., subset VII). Eight of the 15 subsets show exclusive sets of RAG1 haplotypes (subsets "blanci", "enki", "decaryi", "leucocephalus", III, VI, VII and VIII), whereas the seven remaining ones are characterised by a variable degrees of haplotype sharing (between subsets I,II and V, II and "boulengeri", IV and V, and between IX and "verrucosus").

Morphological differentiation

Descriptive statistics are summarised in Table 1 (corresponding raw data available in Supplementary document 2). The two most discriminant traits (SVL and relative tibia length) allow us to morphologically distinguish several of the subsets inferred from the mtDNA data, but other subsets such as "thelenae" or the subsets II and III present an extensive phenotypic variability that prevent their differentiation from several other subsets (Fig. 6A,
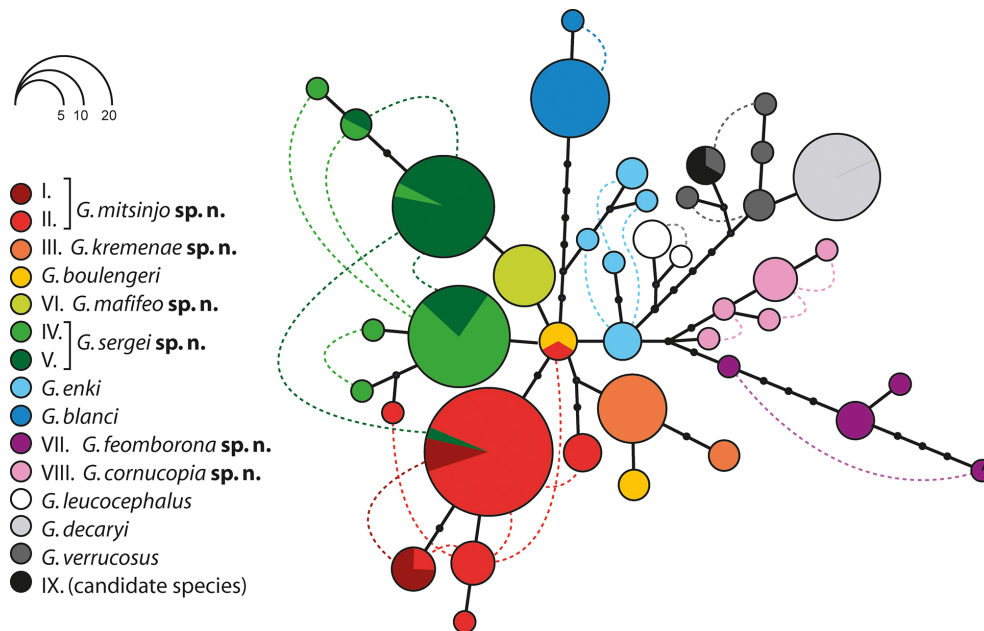

Figure 5. Haplotype network of *Gephyromantis* inferred from the phased DNA sequences of the RAG1 gene. Circles represent haplotypes inferred by phasing, with size proportional to their frequency in the individuals sequenced. Black crossbars indicate the number of mutational steps between haplotypes. The colours assigned to the different clades in the 16S tree are reported on the haplotypes of the corresponding specimens to facilitate comparisons. Coloured dashed curves represent connections between distinct haplotypes found co-occurring in heterozygous individuals.
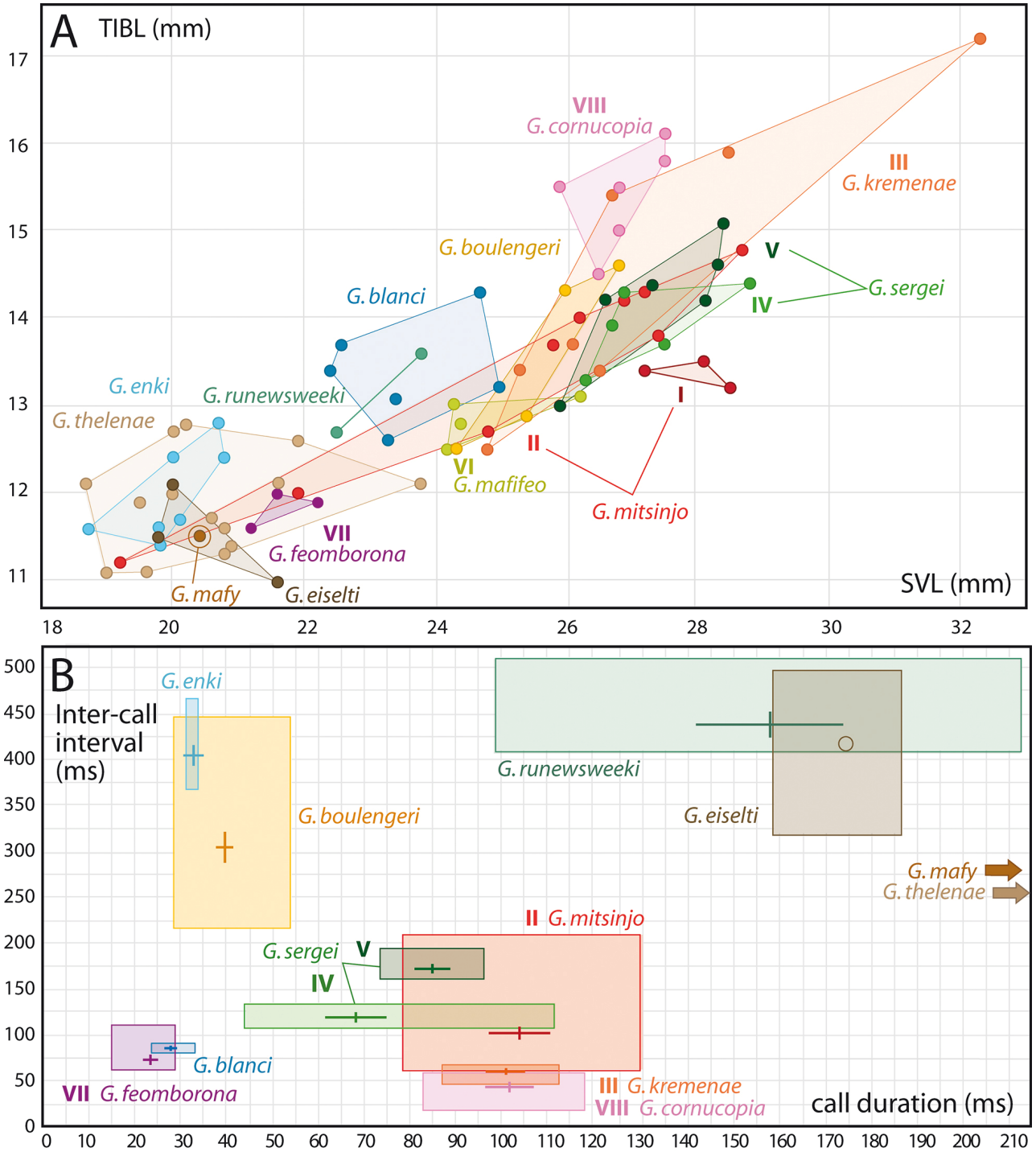
Figure 6. Overview of the morphological and bioacoustic differentiations among the *Gephyromantis blanci*, *G. boulengeri* and *G. eiselti* species complexes. (A) Morphological differentiation illustrated by a bivariate plot of the SVL and TIBL (values in mm; n = 81 genotyped specimens). (B) Bioacoustic differentiation illustrated by a bivariate plot of the call and inter-call durations (min–max values range indicated by rectangles, and mean values and standard deviation by crosses, except for *G. eiselti*; values in ms). Calls of *G. mafifeo* sp. n. and the subset I of *G. mitsinjo* have never been recorded so far. Calls of *G. mafy* and *G. thelenae* are not shown, as their duration is always above 233 ms: call duration of 233–321 ms (mean: 258 ms) with an inter-call interval of 601–1359 ms (807 ms), and call duration of 482–684 ms (mean: 577 ms) with inter-call interval of 1027–1837 ms (1405 ms), respectively.

see also Supplementary document 3 for the same plot, excluding females). Additional PCAs involving the whole set of traits were also carried out (Supplementary document 4), but as they failed to provide significant improvements in terms of subset discrimination, we chose to focus the comparisons mainly on the SVL/TIBL plots, two variables whose morphological meaning is intuitively accessible and easy to verify in the field.

The two focal species complexes (*G. blanci* complex and *G. boulengeri* complex) cannot be sharply differentiated from each other, but subsets forming the *G. boulengeri* complex clearly tend to be larger in size and morphologically less differentiated from each other than those forming the *G. blanci* complex.

Within the *G. boulengeri* complex, the pairs of subsets that can be unambiguously distinguished (i.e. no overlapping of the SVL/TIBL scatterplots) are the following three:

(1) subset I from subsets II, III, IV, V, VI and "boulengeri"; (2) "boulengeri" from IV and V; (3) VI from subsets IV and V. The pairs of subsets that cannot be distinguished are the following: subset II from subsets III, IV, V, VI and "boulengeri"; subset III from IV, V, VI and "boulengeri", and subset IV from V. Overall, subsets II and III are most variable, and least distinguishable from one another.

Within the *G. blanci* complex, all pairs of subsets can be unambiguously distinguished based on the SVL/TIBL plot, except the pair "runewsweeki"/"blanci" (which can nevertheless be unambiguously distinguished by a PCA involving all traits; Supplementary document 4).

Additionally, subsets forming the *G. eiselti* species complex appear to be very weakly differentiated from a morphometric point of view (whether based on the present bivariate analysis or the complementary PCA analyses).

Table 1. Phenotypic variation among the studied species of *Gephyromantis*. Measurements: snout–vent length (SVL); maximum head width (HW); head length from tip of snout to posterior edge of snout opening (HL); horizontal tympanum diameter (TD); horizontal eye diameter (ED); distance between anterior edge of eye and nostril (END); distance between nostril and tip of snout (NSD); distance between both nostrils (NND); forelimb length, from limb insertion to tip of longest finger (FORL); hand length, to the tip of the longest finger (HAL); hind limb length, from the cloaca to the tip of the longest toe (HIL); foot length (FOL); foot length including tarsus (FOTL); and tibia length (TIBL); (in mm, n = number of specimens examined), and call duration (CD) and inter-call interval (ICI) for bioacoustic traits (in ms). The full table of individual measurements is available in Supplementary document 2.

Part 1/2	SVL	HW	HL	TD	ED	END	NSD	NND
	$\overline{x} \pm SD$ min–max	$\overline{x} \pm SD$ min–max	$\overline{x} \pm SD$ min–max	$\overline{x} \pm SD$ min–max	$\overline{x} \pm SD$ min–max	$\overline{x} \pm SD$ min–max	$\overline{x} \pm SD$ min–max	$\overline{x} \pm SD$ min–max
G. mitsinjo (n = 12)	26.0 ± 2.0 19.2–28.7	9.0 ± 0.7 6.4–10.0	9.8 ± 0.6 8.3–10.7	2.2 ± 0.2 1.7–2.5	3.4 ± 0.4 2.7–4.0	2.7 ± 0.3 2.2–3.0	1.8 ± 0.2 1.2–2.2	2.5 ± 0.2 2.0–2.7
G. kremenae (n = 7)	27.2 ± 1.8 24.8–32.3	9.4 ± 0.6 8.3–11.0	11.0 ± 0.6 9.9–12.5	2.4 ± 0.2 1.9–2.6	3.6 ± 0.3 2.9–4.3	3.0 ± 0.2 2.6–3.5	2.0 ± 0.1 1.6–2.3	2.6 ± 0.1 2.3–2.9
G. boulengeri (n = 4)	25.6 ± 0.8 24.3–26.8	8.5 ± 0.3 8.0–8.9	10.4 ± 0.4 10.0–11.0	2.1 ± 0.1 2.0–2.3	3.9 ± 0.2 3.5–4.0	2.9 ± 0.2 2.6–3.3	1.8 ± 0.3 1.4–2.2	2.5 ± 0.2 2.2–2.9
G. sergei (n = 11)	27.3 ± 0.8 25.9–28.8	9.4 ± 0.3 8.6–10.0	10.7 ± 0.5 9.7–12.0	2.5 ± 0.2 2.0–3.1	3.7 ± 0.1 3.2–4.0	2.9 ± 0.2 2.6–3.4	2.0 ± 0.1 1.8–2.2	2.6 ± 0.1 2.4–3.0
G. mafifeo (n = 4)	24.8 ± 0.7 24.2–26.2	8.1 ± 0.3 7.5–8.4	9.6 ± 0.5 8.9–10.0	2.4 ± 0.2 2.2–2.5	3.4 ± 0.1 3.3–3.5	2.8 ± 0.4 2.3–3.3	1.8 ± 0.2 1.6–2.0	2.4 ± 0.2 2.1–2.6
G. enki (n = 7)	20.0 ± 1.1 18.7–21.6	6.8 ± 0.3 6.0–7.6	8.7 ± 0.6 7.7–9.8	1.4 ± 0.1 1.0–1.6	2.8 ± 0.2 2.4–3.3	2.2 ± 0.2 1.9–2.5	1.2 ± 0.2 1.0–1.7	2.0 ± 0.1 1.8–2.4
G. blanci (n = 6)	23.6 ± 0.9 22.4–25.0	7.7 ± 0.2 7.2–8.0	9.3 ± 0.3 8.8–10.0	2.0 ± 0.1 1.9–2.2	3.1 ± 0.1 3.0–3.3	2.6 ± 0.2 2.2–2.9	1.5 ± 0.1 1.4–1.7	2.5 ± 0.1 2.3–2.7
G. cornucopia (n = 6)	26.8 ± 0.4 25.9–27.5	8.3 ± 0.1 8.0–8.5	10.1 ± 0.2 9.8–10.3	2.1 ± 0.1 2.0–2.2	3.4 ± 0.1 3.1–3.6	2.6 ± 0.2 2.4–2.8	1.8 ± 0.1 1.6–2.0	2.7 ± 0.1 2.4–2.9
G. feomborona (n = 3)	21.7 ± 0.4 21.2–22.2	6.7 ± 0.3 6.5–7.1	8.6 ± 0.4 8.1–9.2	1.8 ± 0.1 1.7–1.9	2.9 ± 0.3 2.5–3.3	2.2 ± 0.1 2.0–2.3	1.8 ± 0.2 1.5–2.0	2.0 ± 0.1 1.9–2.1
G. runewsweeki (n = 2)	23.2 ± 0.7 22.5–23.8	7.2 ± 0.3 6.9–7.4	8.6 ± 0.1 8.5–8.7	1.4 ± 0.0 1.4–1.4	2.8 ± 0.1 2.7–2.9	2.1 ± 0.1 2.0–2.1	1.6 ± 0.1 1.5–1.7	2.2 ± 0.2 2.0–2.4
G. mafy (n = 1)	20.4 –	6.4 –	7.7 –	1.4 –	2.9 –	2.0 –	1.3 –	2.1 –
G. thelenae (n = 14)	20.5 ± 1.0 18.7–23.8	6.7 ± 0.3 6.2–7.5	8.4 ± 0.4 7.8–9.7	1.4 ± 0.1 1.2–2.0	2.9 ± 0.2 2.6–3.5	2.2 ± 0.2 1.7–2.6	1.5 ± 0.2 1.1–2.0	2.1 ± 0.1 1.9–2.5
G. eiselti (n = 3)	20.5 ± 0.8 19.8–21.6	6.2 ± 0.3 5.8–6.6	7.7 ± 0.2 7.4–7.9	1.1 ± 0.1 1.0–1.2	2.9 ± 0.2 2.7–3.1	1.9 ± 0.2 1.6–2.3	1.4 ± 0.1 1.2–1.5	1.9 ± 0.0 1.9–2.0

Table 1 continued

Part 2/2	FORL	HAL	HIL	FOTL	FOL	TIBL	CD	ICI
	x̄ ± SD min–max	x̄ ± SD min–max	x̄ ± SD min–max	x̄ ± SD min–max	x̄ ± SD min–max	x̄ ± SD min–max	x̄ ± SD min–max	x̄ ± SD min–max
G. mitsinjo (n = 12)	15.8 ± 1.0 12.5–17.8	7.8 ± 0.5 5.9–8.4	42.6 ± 2.2 34.8–45.9	18.8 ± 1.1 15.0–21.0	11.9 ± 0.8 9.0–13.2	13.4 ± 0.8 11.2–14.8	104.7 ± 13.1 78–130	101.8 ± 33.0 64–212
G. kremenae (n = 7)	17.6 ± 2.0 15.0–22.4	8.3 ± 0.8 7.2–9.9	45.6 ± 4.6 39.0–54.0	19.7 ± 1.8 17.2–23.3	13.9 ± 3.1 10.9–24.6	14.5 ± 1.4 12.5–17.2	101.7 ± 8.2 86–112	61.1 ± 7.8 49–70
G. boulengeri (n = 4)	16.4 ± 1.1 14.4–18.2	7.7 ± 0.5 6.7–8.3	43.7 ± 3.1 39.8–47.7	19.0 ± 1.2 17.3–20.3	12.7 ± 0.8 11.4–13.5	13.6 ± 0.9 12.5–14.6	49.1 ± 3.4 28–54	381.1 ± 40.2 217–449
G. sergei (n = 11)	16.8 ± 0.7 14.7–18.0	9.1 ± 1.7 7.7–18.5	45.0 ± 1.3 43.2–47.3	19.9 ± 0.6 18.3–21.2	12.7 ± 0.7 10.7–13.9	14.1 ± 0.5 13.0–15.1	71.6 ± 26.3 44–112	126.8 ± 24.1 106–196
G. mafifeo (n = 4)*	15.4 ± 0.5 14.9–16.4	7.2 ± 0.4 6.6–7.7	40.4 ± 0.7 39.3–41.3	17.1 ± 0.5 16.0–17.6	11.2 ± 0.2 11.0–11.4	12.9 ± 0.2 12.5–13.1	– –	– –
G. enki (n = 7)	11.0 ± 1.8 5.6–16.5	6.9 ± 1.5 5.1–13.2	34.6 ± 2.9 32.3–44.1	16.0 ± 1.2 14.7–20.3	9.7 ± 0.8 9.0–12.1	12.0 ± 0.6 11.4–13.6	33 31–34	401 371–466
G. blanci (n = 6)	16.7 ± 2.8 14.6–25.0	7.4 ± 0.2 7.1–7.7	42.7 ± 0.9 40.8–43.8	18.9 ± 0.4 18.3–19.7	12.0 ± 0.2 11.6–12.5	13.4 ± 0.4 12.6–14.3	28.1 ± 2.6 24–33	82.5 ± 3.5 78–87
G. cornucopia (n = 6)	16.2 ± 0.6 14.8–17.3	8.0 ± 0.3 7.6–8.5	48.3 ± 1.4 46.3–50.5	22.1 ± 0.5 20.5–23.0	16.2 ± 2.7 13.3–24.4	15.4 ± 0.4 14.5–16.1	102.8 ± 10.8 83–118	42.8 ± 10.7 21–62
G. feomborona (n = 3)	13.8 ± 0.3 13.4–14.2	6.6 ± 0.2 6.5–6.9	37.4 ± 0.6 36.8–38.3	16.7 ± 0.2 16.4–17.0	10.3 ± 0.1 10.2–10.5	11.8 ± 0.2 11.6–12.0	22.7 ± 3.7 15–29	74.9 ± 14.6 58–109
G. runewsweeki (n = 2)	15.2 ± 1.4 13.8–16.5	7.1 ± 0.4 6.7–7.4	42.6 ± 1.6 41.0–44.1	19.4 ± 1.0 18.4–20.3	12.0 ± 0.1 11.8–12.1	13.2 ± 0.5 12.7–13.6	157.9 ± 31.4 97–212	437.2 ± 27.7 411–510
G. mafy (n = 1)	12.7 –	6.2 –	35.1 –	15.7 –	9.4 –	1.5 –	258 ± 24 233–321	807 ± 258 601–1359
G. thelenae (n = 14)	13.3 ± 0.5 12.0–14.6	6.3 ± 0.2 5.7–7.1	35.3 ± 1.3 32.5–37.6	16.0 ± 0.5 15.3–18.5	9.7 ± 0.4 8.1–10.9	11.9 ± 0.5 11.1–12.8	577 482–684	1405 1027–1837
G. eiselti (n = 3)	12.0 ± 0.4 11.7–12.7	6.3 ± 0.3 6.0–6.8	35.9 ± 0.2 35.7–36.3	16.6 ± 0.4 16.0–17.2	9.9 ± 0.5 9.2–10.7	11.5 ± 0.4 11.0–12.1	174 158–186	413 321–499

Bioacoustic differentiation

Recordings of vocalisations within the *Gephyromantis boulengeri* and *G. blanci* species complexes are sparse (Figs 6B, 7, 8, Table 1). Only a few recordings became available across the distributional range of these groups of frogs, most being of limited recording quality and short in duration. One reason for that is possibly the male habit of calling from scattered positions within the rainforest, not forming choruses and not being close to any water body, making it difficult to detect and approach a calling male for recordings. Nevertheless, it has been demonstrated that advertisement calls among these groups of frogs can yield reliable diagnostic characters to differentiate species (e.g., GLAW & VENCES 2002, VENCES & DE LA RIVA 2007, VIEITES et al. 2012, WOLLENBERG et al. 2012). However, the bioacoustic data available and analysed herein proved to be barely conclusive in distinguishing all closely related subsets in the *G. boulengeri* complex based on the call alone. Calls among certain clades are rather similar with respect to pulsatile note structure, call (= note) duration, emission of these calls in call series, and a relatively narrow prevalent bandwidth. There are quantitative differences in inter-call intervals and thus call repetition rate, but given the limited recordings available,

it partly remains unclear if these differences actually correspond to species-specific call differences. However, some general differences nevertheless became evident.

Within the *G. boulengeri* complex (Fig. 7) calls of *G. boulengeri* differ from all subsets for which call records were available (i.e. subsets II, III, IV, V, records missing for subsets I and VI) by quantitative temporal call parameters, namely shorter call duration (28–54 vs. 74–130 ms) and distinctly longer inter-call intervals within call series (217–449 vs. 49–212 ms; maximum value referring to a single measurement in calls from subset II). One slight exception is the calls of the subset IV from Ifanadiana showing a similar call duration (44–56 ms), but still significantly shorter inter-call intervals (106–133 ms). Call differences among the four candidate species are far more subtle, as temporal parameters exhibit broad overlap among the clades. Calls of the subset II might differ from III, IV and V by the acceleration of call rate within series, a qualitative trait not observed in the calls of the other clades. Calls of subset V exhibit longer inter-call intervals compared to calls of III and IV (163–196 vs. 106–136 ms). Available data are insufficient to classify the quantitative call differences revealed among the subsets III, IV, and V as representing constant respective characters (Table 2).

Within the *G. blanci* complex (Fig. 8), call differences are much more pronounced. The call of the subset "blanci" is characterised by short series always containing 5 calls only, distinguishing it from all other calls analysed herein. Similarly, calls of "runewsweeki" are unique among the calls studied in being multi-note calls, in contrast to all other
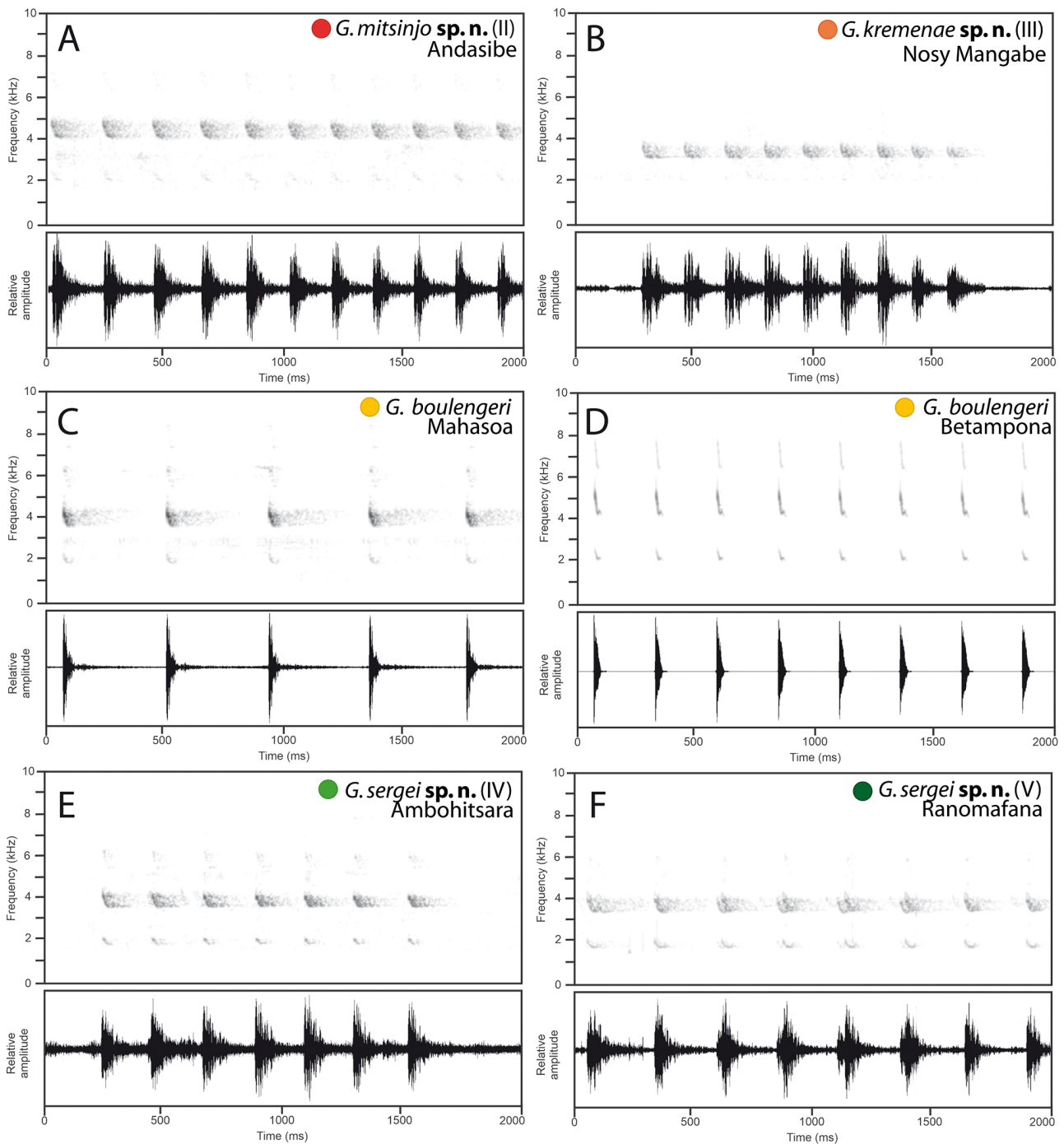

Figure 7. Audiospectrograms and corresponding oscillograms at 2000 ms time scale within the *Gephyromantis boulengeri* species complex: (A) *G. mitsinjo* sp. n. (subset II) from Andasibe (11 calls figured, recording high-pass filtered at 1500 Hz); (B) *G. kremenae* sp. n. (subset III) from Nosy Mangabe (9 calls, high-pass filtered at 2000 Hz); (C) *G. boulengeri* (subset "boulengeri") from Mahasoa (5 calls, high-pass filtered at 1000 Hz), and (D) same species from Betampona (8 calls, high-pass filtered at 1500 Hz); (E) *G. sergei* sp. n. (subset IV) from Ambohitsara (7 calls, high-pass filtered at 1500 Hz), and (F) same species (subset V) from Ranomafana (8 calls, band-pass filtered at 1500–6250 Hz; note that except for the last two calls in this figure, another conspecific male is calling in the background causing some overlap of calls).

calls being single-note calls according to the terminological scheme and methods used herein. Calls of the subset VII are characterised by a rather short call duration (15–29 ms) and the highest recorded call repetition rate within call series (520–660 calls/minute), whereas calls tentatively allocated to subset VIII are characterised by containing the shortest recorded inter-call intervals (21–62 ms) among the calls analysed. These differences in character and parameters are at a level typical for species-specific bioacoustic differentiation (Table 3; see Köhler et al. 2017). The same is true for calls among clades in the *G. eiselti* complex, which all exhibit distinct species-specific call differences (Table 3).

Taxonomic conclusions
Integration of multiple lines of evidences

To reduce the complexity of the dataset, we combined two pairs of subsets in the *G. boulengeri* species complex for which the distinctiveness at the species level is not unambiguously supported. This applies to (1) the subsets IV and V (henceforth regarded as a new subset "IV+V") which show an extensive RAG1 haplotype admixture, with no evidence of morphological differentiation, inconclusive call differentiation, and are found in sympatry in Ranomafana, and (2) the subsets I and II (henceforth "I+II") which also show an important haplotype sharing in RAG1 (no call available for A). We are aware that combining subsets IV and V (while recognizing subset VI as distinct species) may appear illogical, mainly because the mitochondrial tree suggests a paraphyly of IV+V. It is likely that the IV+V cluster of individuals is composed of two species, but with the limited data at hand and taking into account the possibility of mitochondrial introgression, it is difficult to assess which of the sampled individuals and populations may belong to either species or to hybrids, respectively; further data, preferably based on phylogenomic approaches, will be needed to disentangle the taxonomy of the IV+V populations.
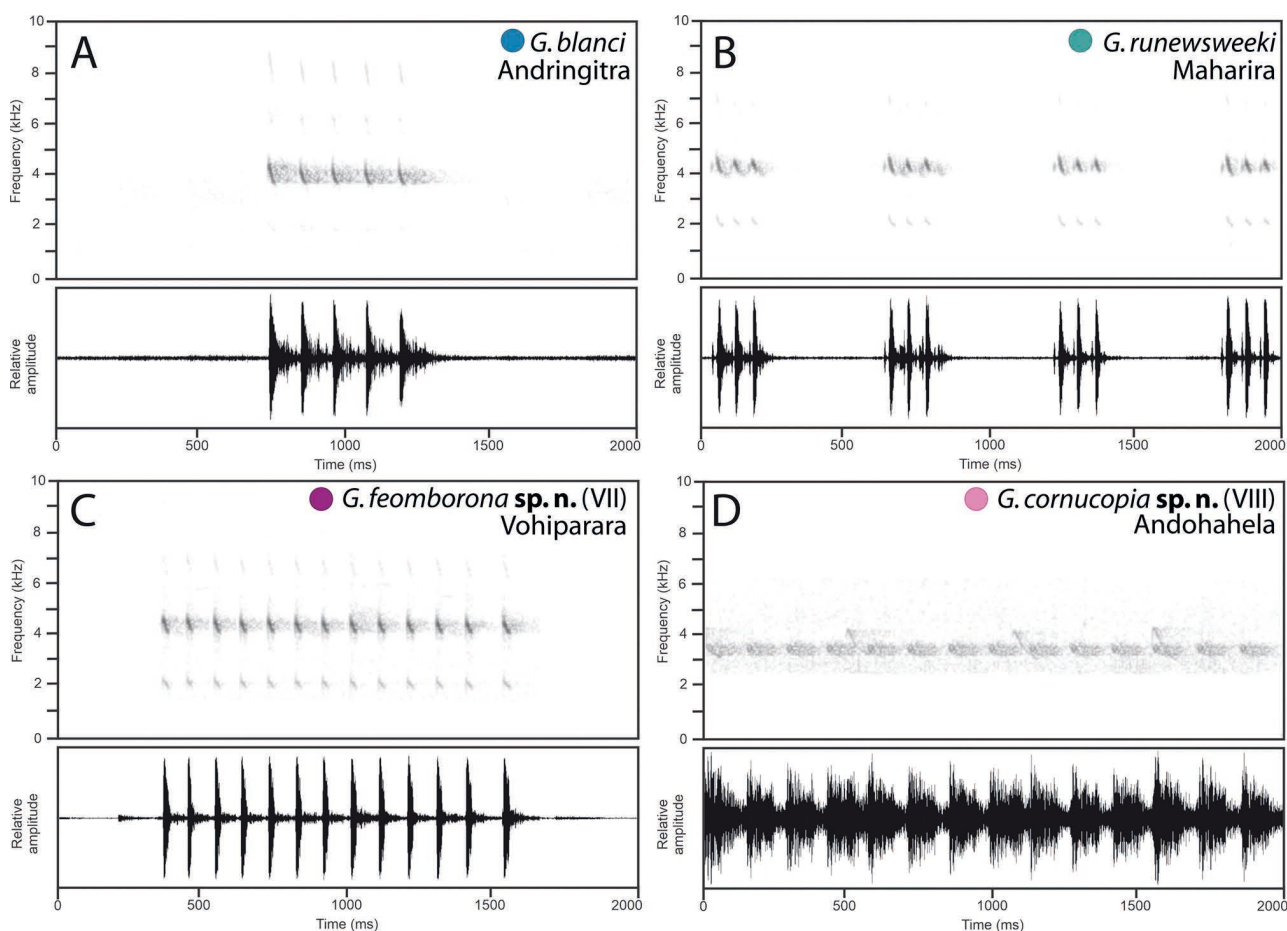

Figure 8. Audiospectrograms and corresponding oscillograms at 2000 ms time scale of advertisement calls within the *Gephyromantis blanci* species complex: (A) *G. blanci* (subset "blanci") from Andringitra (5 calls figured, recording high-pass filtered at 1000 Hz); (B) *G. runewsweeki* (subset "runewsweeki") from Maharira (4 calls, high-pass filtered at 1000 Hz); (C) *G. feomborona* sp. n. (subset VII) from Vohiparara (13 calls, high-pass filtered at 1500 Hz); and (D) calls tentatively assigned to *G. cornucopia* sp. n. (subset VIII) from Andohahela (14 calls, band-pass filtered at 2500–6000 Hz).

Table 2. Summary of the different lines of evidence in favor of the specific distinctiveness for each pair of subsets with the *G. boulengeri* species complex: Monophyly (yes/no: recovered or not as distinct clades on the 16S tree), 16S p-distances (min–max range, %), nDNA haplotype exclusivity based on RAG1 (yes/no), morphological differentiation based on morphometry (yes: ***unambiguously distinct scatterplots separated by a gap, *scatterplots well differentiated, but in contact or barely overlapping, no: scatterplots significantly overlapping). Bioacoustic differentiation (yes: ***significantly distinct, *weak to moderate differentiation, no). Known occurrences of sympatry (records in an area of ca. < 5 km, yes/no). N/A: missing data. [1] significant haplotype sharing. [2] weak haplotype sharing. [3] subset VI nested within subset (IV+V).

Subsets	III	*boulengeri*	(IV+V)	VI
Subset (I+II)				
monophyly	yes	yes	yes	yes
p-distances	3.1–6.9	3.1–6.6	4.4–9.7	5.5–7.1
haplo. excl.	yes	no[1]	no[2]	yes
morphology	no	no	no	no
bioacoustic	yes*	yes***	yes*	N/A
sympatry	no	no	no	no
subset III				
monophyly		yes	yes	yes
p-distances		1.9–4.6	5.3–9.8	6.3–9.2
haplo. excl.	—	yes	yes	yes
morphology		no	no	no
bioacoustic		yes***	no	N/A
sympatry		no	no	no
boulengeri				
monophyly			yes	yes
p-distances			4.7–12.5	5.1–8.1
haplo. excl.		—	yes	yes
morphology			yes***	no
bioacoustic			yes***	N/A
sympatry			no	no
subset (IV+V)				
monophyly				no[3]
p-distances				4.8–9.1
haplo. excl.			—	yes
morphology				yes***
bioacoustic				N/A
sympatry				no

The different lines of evidence in favor (or against) the specific distinctiveness for each resulting pair of subsets are summarised in Table 2 for the *G. boulengeri* species complex and Table 3 for the *G. blanci* and *G. eiselti* complexes. Our data suggest the presence of at least 17 lineages in the subgenus *Gephyromantis* that merit species status. A total of 11 scientific nomina have so far been coined to refer to populations of this group of frogs. Based on Glaw & Vences (2002) for *G. enki*, Vences & De la Riva (2007) for *G. runewsweeki*, Wollenberg et al. (2012) for *G. decaryi*, *G. hintelmannae* and *G. verrucosus*, and Vieites et al. (2012) for *G. enki*, *G. eiselti*, *G. mafy* and *G. thelenae*, the identity of most nomina in the subgenus *Gephyromantis* has been recently revised and tied to DNA sequences and

advertisement call data. Given that no junior synonyms of any species in the subgenus *Gephyromantis* exist (Frost 2022), no earlier names possibly applying to any of the lineages identified herein need to be taken into account. This leaves only the names *Gephyromantis decaryi leucocephala* Angel, 1930 (currently *Gephyromantis leucocephalus*), *Gephyromantis blanci* Guibé, 1973, and *Gephyromantis boulengeri* Methuen, 1920, in need of precise definition.

For *G. leucocephalus,* our data suggest a need for further revision due to the substantial genetic variation observed among populations assigned to this species. However, based on morphological data presented by Glaw & Vences (2002) and the origin of the syntype series (from two low-elevation sites in the South East, Befotaka and Midongy du Sud), its membership to the south-eastern genetic clade here considered under this name is unambiguous. A more precise analysis of *G. leucocephalus* is pending, and ideally should include genetic data from topotypical material.

For *G. blanci,* in various field explorations of the high-elevation forests of the Andringitra Massif, we found only one species of the subgenus *Gephyromantis*; herein we provide a genetic and bioacoustic characterisation of this species, and evidence for its morphological agreement with the *blanci* holotype.

In contrast, ascertaining the identity of *G. boulengeri* is a more convoluted task. This species was described from the locality "Folohy" whose exact geographical placement is unknown. A detailed discussion of this site is provided by Vences et al. (2022) who, along with Blommers-Schlösser & Blanc (1991), conclude that Folohy refers to a low- or mid-elevation site close to Toamasina in the Northern Central East (see Rosa et al. 2012). Unfortunately, our attempts to obtain genetic data of the *boulengeri* holotype through a 'barcode fishing' approach (e.g., Rancilhac et al. 2020, Scherz et al. 2020) failed, but two other *Gephyromantis* type specimens from Folohy could be assigned by this approach: *G. luteus* and *G. malagasius* (see Vences et al. 2021a, 2022). While *G. malagasius* turned out to be a rarely collected species whose phylogeographic structure is unknown, the type of *G. luteus* clustered very closely to specimens of *G. luteus* from Betampona, a site close to Toamasina. This confirms that the Folohy collecting locality was in the Toamasina region, and probably very close to Betampona. From this general area, and from Betampona specifically, our collections only yielded representatives of one lineage of the subgenus *Gephyromantis,* which in general morphology agreed with the *G. boulengeri* holotype. Therefore, we consider this lineage (present in Betampona and other sites in the Toamasina area) as *G. boulengeri.*

Having thus assigned all 11 available scientific names in the subgenus to genetic lineages, eight deep lineages remain unclassified. As explained above, for two pairs of these (subsets I and II, and subsets IV and V, respectively) we consider the available data as insufficient for a conclusive integrative species delimitation, and therefore, in a taxonomically conservative approach, consider them for the time being as representing only two distinct unde-

Table 3. Summary of the different lines of evidence in favor of the specific distinctiveness for each pair of subsets with the *G. blanci* and *G. eiselti* species complexes: Monophyly (yes/no: recovered or not as distinct clades on the 16S tree), 16S p-distances (min–max range, %), nDNA haplotype exclusivity based on RAG1 (yes/no), morphological differentiation based on morphometry (yes: ***unambiguously distinct scatterplots separated by a gap, *scatterplots well differentiated, but in contact or barely overlapping, no: scatterplots significantly overlapping). Bioacoustic differentiation (yes: ***significantly distinct, *weak to moderate differentiation, no). Known occurrences of sympatry (records in an area of ca. < 5 km, yes/no). N/A: missing data.

Subsets	VIII	*runewsweeki*	*enki*	*thelenae*	*mafy*	*eiselti*	*blanci*
subset VII							
monophyly	yes	yes	yes	yes	yes	yes	yes
p-distances	5.1–7.3	5.2–6.6	5.2–7.7	7.7–8.3	7.4–9.0	6.1–7.8	4.3–6.4
haplo. excl.	yes	N/A	yes	N/A	N/A	N/A	yes
morphology	yes***	yes***	yes***	no	yes***	yes***	yes***
bioacoustic	yes***	yes***	yes***	yes***	yes***	yes***	yes***
sympatry	no	yes	yes	no	no	no	no
subset VIII							
monophyly		yes	yes	yes	yes	yes	yes
p-distances		8.4–8.6	7.1–8.9	8.2–9.2	8.0–9.2	7.4–8.5	6.7–7.5
haplo. excl.	—	N/A	yes	N/A	N/A	N/A	yes
morphology		yes***	yes***	yes***	yes***	yes***	yes***
bioacoustic		yes***	yes***	yes***	yes***	yes***	yes***
sympatry		no	no	no	no	no	yes
runewsweeki							
monophyly			yes	yes	yes	yes	yes
p-distances			4.1–4.3	7.9–7.9	8.6–8.6	7.9–7.9	3.4–3.9
haplo. excl.		—	N/A	N/A	N/A	N/A	N/A
morphology			yes***	yes***	yes***	yes***	yes***
bioacoustic			yes***	yes***	yes***	yes***	yes***
sympatry			yes	no	no	no	no
enki							
monophyly				yes	yes	yes	yes
p-distances				8.0–9.2	9.0–9.4	7.8–8.9	2.4–4.1
haplo. excl.			—	N/A	N/A	N/A	yes
morphology				no	yes***	yes***	yes***
bioacoustic				yes***	yes***	yes***	yes***
sympatry				no	no	no	no
thelenae							
monophyly					yes	yes	yes
p-distances					3.6–3.6	2.3–2.3	7.1–8.0
haplo. excl.				—	N/A	N/A	N/A
morphology					no	yes*	yes***
bioacoustic					yes***	yes***	yes***
sympatry					no	no	no
mafy							
monophyly						yes	yes
p-distances						2.9–2.9	6.7–8.8
haplo. excl.					—	N/A	N/A
morphology						yes***	yes*
bioacoustic						yes*	yes*
sympatry						no	no
eiselti							
monophyly							yes
p-distances							5.9–7.6
haplo. excl.						—	N/A
morphology							yes*
bioacoustic							yes*
sympatry							no

scribed species composed each of two deep conspecific lineages sensu Vieites et al. (2009), i.e. "I+II" and "IV+V", respectively. In conclusion, six distinct species-level lineages are formally named and described as new species in the following.

Taxonomic species accounts
Gephyromantis boulengeri species complex

Gephyromantis boulengeri Methuen, 1920
Figs 2, 3, 4–7, 9, 10

Remarks. Referred to as *Gephyromantis* sp. aff. *boulengeri* [Ca FJ559196], *G.* sp. 25 or *G.* sp. Ca25 in previous studies, and earlier as subset *"boulengeri"* in the present work

Holotype. TM 10876 (formerly 1013), according to Blommers-Schlösser & Blanc (1991). Type locality: "Folohy, East Madagascar" (Fig. 9).

Material examined (n = 4). ZSM 1878/2008 (ZCMV 8089; call voucher), ZSM 1879/2008 (ZCMV 8820), two adult males, from Mahasoa campsite near Ambodisakoa village (17.2977° S, 48.7020° E, 1032 m a.s.l.), NE Vohimena, NE Lake Alaotra, Madagascar, collected on 13 February 2008 by D. R. Vieites, J. Patton, P. Bora, and M. Vences; ZSM 652/2009 (ZCMV 8905), unsexed adult, Ankanin'ny Nofy (18.5926° S, 44.2387° E, 19 m a.s.l.), Madagascar, collected on 9 April 2009 by P.-S. Gehring, F. M. Ratsoavina,

and E. Rajeriarison; ZSM 653/2009 (ZCMV 8909), male adult, from Vohibola (18.5936° S, 49.2375° E, 14 m a.s.l.), Madagascar, collected on 10 April 2009 by P.-S. Gehring, F. M. Ratsoavina, and E. Rajeriarison.

Diagnosis. A member of the subgenus *Gephyromantis* in the genus *Gephyromantis* on the basis of (1) presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), (2) small size (SVL < 35 mm), (3) slightly enlarged terminal discs of fingers, (4) presence of outer metatarsal tubercle, (5) absence of webbing on hands and presence of only rudimentary webbing on feet, (6) tight connection of tissue surrounding the two lateral metatarsalia, (7) presence of femoral glands in males, (8) presence of paired/bilobed blackish vocal sacs in males, (9) diurnal calling behaviour not concentrated at water bodies, (10) molecular phylogenetic relationships.

Distinguished from all nominal species within the subgenus by combination of (1) moderate size compared to other species (male SVL up to at 26.8 mm), (2) dorsum with coarse granules, (3) upper lip typically with alternating white/dark brown markings, (4) lower lip ventrally without a yellowish tint in life; (5) no reddish tint on ventral side of thighs in life, (6) relatively short call duration of about 28–54 ms with long inter-call intervals of 217–464 ms. This species is furthermore differentiated from all nominal species of *Gephyromantis,* except *G. kremenae,* by a substantial molecular differentiation, with uncorrected pairwise distances > 3% in the mitochondrial 16S gene.

Figure 9. Dorsal, ventral and lateral views of preserved holotypes of (A) *Gephyromantis blanci* (MNHN 1972.0183, from "Ambalamarovandana"), and (B) *G. boulengeri* (TMP 10876, from "Folohy, East Madagascar").

● *Gephyromantis boulengeri*

Figure 10. Dorsal and ventral views of specimens of *Gephyromantis boulengeri* in life. Individuals from Mahasoa: (A, B) ZSM 1878/2008; (C, D) ZSM 1879/2008; and from Betampona: (E*, F) MRSN A6345; (G, H) FAZC 13882, uncatalogued specimen. *Asterisk indicates mirror reversed picture.

The species is most similar to *G. mitsinjo* sp. n. and *G. kremenae* sp. n. (described below) which are also characterised by the presence of coarse granules on the dorsum and similar body size. However, *G. boulengeri* is distinguished from *G. mitsinjo* by its lower note repetition rate (116–235 vs. 280–345 calls/min), a shorter call duration (about 28–54 vs. 78–130 ms) and a longer inter-call interval (217–464 vs. 64–212 ms) in advertisement calls; and differs from *G. kremenae* by lower note repetition rate (116–235 vs. 390 calls/min), a shorter call duration (about 28–54 vs. 86–112 ms) and longer inter-call intervals (217–464 vs. 49–70 ms). For a distinction of other new species described herein, see diagnoses in the respective species accounts below.

Redescription of type material. *Gephyromantis boulengeri* was described by Methuen (1920) based on two specimens in the Transvaal Museum (Pretoria), the female holotype TM 10876 (formerly TM Rept. 1013) and the paratype TM 10875 (formerly TM Rept. 1012). We here present photographs of the holotype (Fig. 9), as well as measurements of this specimen and of the paratype TM 10875 (Supplementary document 2). Based on this, *G. boulengeri* can be characterised as follows: The female holotype of *G. boulengeri* is in a fairly good state of preservation. Our examination and the photos of the holotype revealed, among others, the following traits: body slender, head slightly wider than body, snout rounded in dorsal and lateral view; nostrils directed posterolaterally, protuberant, canthus rostralis distinct, rounded; loreal region concave; tympanum distinct, rounded, supratympanic fold distinct but weakly developed; arms slender, prominent single subarticular tubercles, inner and outer metacarpal tubercles recognisable; fingers without webbing, finger discs distinctly enlarged. Hindlimbs slender; lateral metatarsalia connected; inner and outer metatarsal tubercles present; basal webbing between toes. Skin on the dorsal surface tuberculated, with discontinuous short ridges; dorsolateral ridges present, weakly developed, interrupted; skin on the lateral sides tuberculated. Ventral skin smooth on throat, chest and limbs, granular on central and posterior belly. After almost a century in preservative, dorsal surfaces brown, with some paler spots and flecks in scapular region and mid-dorsum, upper eyelids dark brown, faint dark brown interorbital bar. Laterally brown, supratympanic fold dark brown; upper lip and tip of snout with diffuse white flecking. Arms and legs dorsally with irregular light and dark transverse bars. Belly cream; throat and chest brown with some scattered cream flecking and narrow cream median line; a row of seven well-defined irregularly shaped cream spots along the lower lip.

Compared to the holotype, the female paratype of *G. boulengeri* has a brown dorsum with almost regularly distributed dark brown spots and a light vertebral stripe (Fig. 2). Its throat is dark brown with a narrow light median line barely recognisable. The skin texture on the dorsum appears far less tuberculated compared to the holotype. Its ratio of head length to head width (HL/HW) is 1.25. Meas-

urements taken of the paratype TM 10875 are as follows (all in mm: SVL, 27.8; HW, 9.0; HL, 11.3; ED, 4.3; TD, 1.8; END, 2.6; NSD, 1.9; NND, 2.8; HAL, 6.1/7.6 (left/right); FORL, 14.5/15.1; HIL, 40.9/43.6; FOL, 12.2/12.1; FOTL, 18.5/17.9; IMTL, 0.5; IMTH, 1.1. Because these measurements were taken by a different person (LdP) than those of the newly collected material (MV), we did not include these measurements in the morphometric comparisons.

Redescription based on fresh material. Based on call voucher ZSM 1878/2008 from Mahasoa. Male specimen in a good state of preservation, on the right thigh a tissue was taken for DNA analyses. SVL 26.0 mm. Body slender, ratio of head length to head width HL/HW = 1.18, head slightly wider than body, snout rounded in dorsal and lateral view; nostrils directed posterolaterally, protuberant, canthus rostralis distinct, rounded; loreal region concave; tympanum distinct, rounded, ratio of tympanum diameter to eye diameter TD/ED = 0.56; supratympanic fold distinct but weakly developed; tongue ovoid, posteriorly bifid; maxillary teeth present; vomerine teeth absent; choanae rounded. Arms slender, distinct single subarticular tubercles; inner and outer metacarpal tubercles recognisable; fingers without webbing; relative length of fingers 1=2<4<3; finger disks distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches the nostril when the hindlimb is adpressed along the body; lateral metatarsalia connected; inner and outer metatarsal tubercles distinct; traces of webbing between toes; relative length of toes 1<2<5<3<4. Skin on the dorsal surface slightly tuberculated, with discontinuous, weakly developed and interrupted dorsolateral ridges in the middle section of the dorsum; skin on the lateral sides tuberculated with additional short ridges. Ventral skin smooth on throat, chest and limbs, granular on central and posterior belly. Femoral glands not visible in external view, consisting of eight large gland granules arranged in a more or less linear series (right thigh only).

After two years in preservative, dorsum between dorsolateral ridges and snout region reddish brown; an indistinct and interrupted dark transverse bar is present between the eyes. Laterally dark brown, with a sharp dorsolateral colour border; irregular black markings are present in the loreal region and below the supratympanic fold; tympanum dark brown, with narrow, lightly coloured anterior and lower margins; upper lip and tip of snout with a diffuse white mottling. Arms and legs dorsally with diffuse light and dark brown mottling. Vocal sacs black. Ventral side dark brown anteriorly, dirty white posteriorly. Throat brown, with a narrow, partly broken, light median line; lighter areas are present towards the ventral insertions of the vocal sacs; a row of seven well-defined irregularly shaped white spots along the lower lip; belly white, with large areas of dark brown mottling towards the chest and lateral sides. Ventral surfaces of forelimbs with a well-defined light median zone; ventral side of thigh white, with some diffuse light brown mottling laterally; ventral shank with a well-defined light median zone.

Habitat, habits, and distribution. At Mahasoa, *G. boulengeri* was relatively common around our camp site, males were heard calling in several places, and juveniles fell in pitfall lines. The site was in a highly disturbed rainforest. Males were calling during the day. In the coastal forest of Ankanin'ny Nofy and the neighbouring Vohibola forest, subadults were found under relatively dry conditions in November 2022 in areas of remaining humidity such as dried temporary swamps and streams, and only a few calling individuals were heard. In contrast, during the rainy season numerous calling males were found and heard on the forest floor of the coastal forests of Ankanin'ny Nofy and Vohibola in April 2009. At Betampona, aggressive territorial behaviour between males of this species have been documented by Lam et al (2020). As currently defined, *G. boulengeri* has been recorded from the following localities: (1) Ankanin'ny Nofy and Vohibola, (2) Betampona (including Piste Sabefohoza, Rendryrendry), (3) Ivoloina Park, (4) Mahasoa, (5) Sahavontsira. Furthermore, a previous record from (6) Moramena probably refers to the Moramena corridor near the Zahamena reserve, and (7) the type locality Folohy is probably located close to Betampona (Fig. 3).

Vocalisation. Advertisement calls recorded on 13 February 2008 at Mahasoa (air temperature not measured) consist of a single pulsatile note of short duration. Calls (= notes) are emitted in call series at rather regular intervals. Slight amplitude modulation is evident in calls with maximum energy being present at the beginning of the call, rapidly decreasing towards its end. Structure of pulses within notes is diffuse, but usually two distinctly separated pulses are recognisable at the beginning of each note, followed by shorter pulses that are partially fused. Numerical parameters of 48 analysed calls from two individuals are as follows: call duration (= note duration) 41–54 ms (49.1 ± 3.4 ms); inter-call interval 330–449 ms (381.1 ± 40.2 ms); dominant frequency 3835–4493 Hz (4135 ± 244 Hz); prevalent bandwidth 3500–4800 Hz. Call series (n = 5) had a duration of 5325–8342 ms (6374 ± 1175 ms) and contained 12–19 calls (15.0 ± 2.7). Call rate within series varied from approximately 116–145 calls/minute (Fig. 7).

Calls recorded on 30 October 2007 at Sahabefoza, Betampona (air temperature 20°C; see Rosa et al. 2011, track 23) generally agree in character with those recorded at Mahasoa, but have a somewhat less distinct pulsatile structure and were repeated at a faster rate. Numerical parameters of 20 analysed calls from two individuals are as follows: call duration (= note duration) 28–35 ms (31.4 ± 1.8 ms); inter-call interval 217–255 ms (229.6 ± 9.4 ms); dominant frequency 4285–4478 Hz (4386 ± 74 Hz); prevalent bandwidth 4000–5700 Hz. Call series (n = 2) had a duration of 3159 and 1641 ms and contained 13 and 7 calls, respectively. Call rate within series varied from approximately 200–235 calls/minute (Fig. 7).

Calls recorded on 22 November 2022 at Ankanin'ny Nofy (estimated air temperature 24°C) consist of a short pulsatile note repeated in call series. Calls generally agree in character with those recorded at Mahasoa, although call duration and inter-call intervals were difficult to measure due to great recording distance and some reverb being apparently involved (thus to be regarded with some reservation). Numerical parameters of 14 analysed calls are as follows: call duration (= note duration) 56–89 ms (71.5 ± 10.4 ms); inter-call interval 306–464 ms (350.7 ± 59.1 ms); dominant frequency 4296–4401 Hz (4331 ± 38 Hz); prevalent bandwidth 4100–5200 Hz. Call series (n = 2) had a duration of 3500 and 6180 ms and contained 9 and 16 calls, respectively. Call rate within series varied from approximately 145–160 calls/minute.

Gephyromantis mitsinjo sp. n.
Fig. 2, 3, 4–7, 11, 12

LSID: urn:lsid:zoobank.org:act:6BA4F387-2E98-4813-A938-A8CF9F5F83E7

Remarks. Referred to as *G.* sp. 24 or Ca24 in previous studies, and earlier as subsets I and II in the present work.

Holotype. ZSM 1830/2008 (ZCMV 8138), adult male, from Andasibe, (18.9229° S, 48.4186° E, ca. 900 m a.s.l.), eastern Madagascar, collected on 27 January 2008 by K. C. Wollenberg and M. Vences. Genotyped as belonging to the subset II (Fig. 11).

Paratypes (n = 26, all assigned to the subset II). ZSM 160/2016 (FGZC 5083), adult female, ZSM 161/2016 (FGZC 5085), adult male, ZSM 162/2016 (FGZC 5082), adult male, all from Vohimana, Sentier Botanique (18.9203° S,
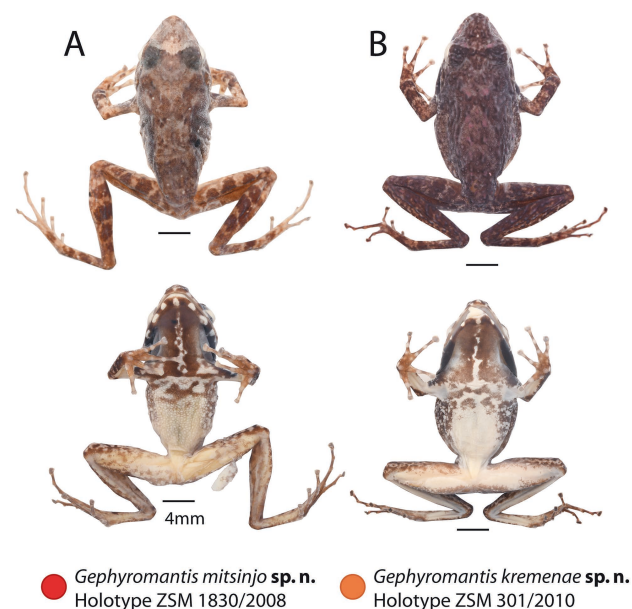

Figure 11. Dorsal and ventral views of preserved holotypes of (A) *Gephyromantis mitsinjo* sp. n. (ZSM 1830/2008), and (B) *G. kremenae* sp. n. (ZSM 301/2010).

48.5160° E, 786 m a.s.l.), collected on 28 December 2015 by F. GLAW, D. PRÖTZEL, and L. RANDRIAMANANA; ZSM 159/2016 (FGZC 4959), adult male, from Vohimana, Pandanus forest (18.9172° S, 48.4930° E, 804 m a.s.l.), Madagascar, collected on 26 December 2015 by F. GLAW, D. PRÖTZEL, and L. RANDRIAMANANA; ZSM 404/2006 (ZCMV 1449), adult female, ZSM 405/2006 (ZCMV 1469), adult male, ZSM 406/2006 (ZCMV 2442) and ZSM 1971/2006 (ZCMV 2437), both unsexed subadults, all from An'Ala Forest camp (18.9193° S, 48.4880° E, 889 m a.s.l.), Madagascar, collected between 7 and 10 February 2006 by D. R. VIEITES, M. VENCES, F. RABEMANANJARA, P. BORA, C. WELDON, and J. PATTON; ZSM 1832/2008 (ZCMV 8142), ZSM 1833/2008 (ZCMV 8143), one male and one unsexed adult specimen, from Besariaka (South of Moramanga), Madagascar, collected on 2 February 2008 by K. C. WOLLENBERG; ZSM 1826/2008 (ZCMV 8004), adult male, and ZSM 1827/2008 (ZCMV 8101), ZSM 1828/2008 (ZCMV 8105), ZSM 1829/2008 (ZCMV 8132), ZSM 1831/2008 (ZCMV 8140), four adult unsexed specimens, all from the exact same locality as holotype, collected between 15 and 27 January 2008 by K. C. WOLLENBERG; ZSM 1846/2008 (ZCMV 8115), ZSM 1847/2008 (ZCMV 8116), both adult specimens, from Camp Prolemur, Marinjo site, near Mantadia (18.7706° S, 48.4291° E, 995 m a.s.l), Madagascar, collected on 20 January 2008 by K. C. WOLLENBERG; ZSM 1871/2008 (ZCMV 8760), ZSM 1872/2008 (ZCMV 8761), ZSM 1873/2008 (ZCMV 8763), all adult specimens, from Camp Prolemur, Marinjo site, near Mantadia (18.7706° S, 48.4291° E, 995 m a.s.l.), Madagascar, collected on 30 January 2008 by L. DU PREEZ, C. WELDON, and L. RAHARIVOLOLONIAINA; ZSM 5059/2005 (ZCMV 999) adult specimen, ZSM 5060/2005 (ZCMV 2204), adult female, ZSM 5061/2005 (ZCMV 2206), adult male, all from Andasibe (pitfall, 18.9362° S, 48.4122° E, 939 m a.s.l.), Madagascar, collected in February 2005 by R. DOLCH and collaborators; ZSM 307/2010 (FGZC 4626), adult specimen, Ambatofotsy (19.5431° S, 48.3165° E, 907 m a.s.l.), Madagascar, collected on 15/16 April 2010 by M. PABIJAN, F. RANDRIANASOLO, and S. RASAMISON; ZSM 153/2006 (FGZC 975), adult male, Andasibe (station forestière, 18.9362° S, 48.4122° E, 939 m a.s.l.), Madagascar, collected on 8 April 2006 by F. GLAW, J. KÖHLER, and H. ENTING; ZSM 306/2010 (FGZC 4475), south of Niagarakely (19.2188° S, 48.2322° E, 1034 m a.s.l.), Madagascar, collected on 14 April 2010 by F. GLAW, J. KÖHLER, P.-S. GEHRING, K. MEBERT, and E. RAJERIARISON.

Additional material (all assigned to the subset I). ZSM 302/2010 (FGZC 4437), ZSM 303/2010 (FGZC 4438), ZSM 304/2010 (FGZC 4441), adult males, from Anosibe An'Ala (19.4349° S, 48.2007° E, 636 m a.s.l.), Madagascar, collected on 13 April 2010 by E. RAJERIARISON and F. GLAW; ZSM 305/2010 (FGZC 4460), adult male, from the forest near Tarzanville (19.3244° S, 48.2199° E, 881 m a.s.l.), Madagascar, collected on 13 April 2010 by F. GLAW, J. KÖHLER, P.-S. GEHRING, K. MEBERT, E. RAJERIARISON, and F. M. RATSOAVINA.

Diagnosis. A member of the subgenus *Gephyromantis* in the genus *Gephyromantis* on the basis of (1) presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), (2) small size (SVL < 35 mm), (3) slightly enlarged terminal discs of fingers, (4) presence of outer metatarsal tubercle, (5) absence of webbing on hands and presence of only rudimentary webbing on feet, (6) tight connection of tissue surrounding the two lateral metatarsalia, (7) presence of femoral glands in males, (8) presence of paired/bilobed blackish vocal sacs in males, (9) diurnal calling behaviour not concentrated at water bodies, (10) molecular phylogenetic relationships.

Distinguished from all nominal species within the subgenus by combination of (1) moderate size compared to other species (male SVL up to at least 27 mm; up to 29 mm in sublineage I), (2) dorsum with coarse granules, (3) upper lip typically with alternating white/dark brown markings, (4) lower lip ventrally without a yellowish tint in life; (5) no reddish tint on ventral side of thighs in life; (6) relatively short hindlimbs (ratio TIBL/SVL 0.50–0.58), (7) call series of about 12–15 calls with 78–130 ms duration, with a high call repetition rate of about 280–345 calls/minute. The new species is furthermore differentiated from all nominal species of *Gephyromantis* by a substantial molecular differentiation, with uncorrected pairwise distances > 3% in the mitochondrial 16S gene.

The species is most similar to *G. boulengeri* and *G. kremenae* sp. n. (described below) which are also characterised by the presence of coarse granules on the dorsum and similar body size. However, *G. mitsinjo* is distinguished from *G. boulengeri* by its higher note repetition rate (280–345 vs. 116–235 calls/min), a longer call duration (about 78–130 vs. 28–54 ms) and a shorter inter-call interval (64–212 vs. 217–464 ms) in advertisement calls; and differs *G. kremenae* by a longer inter-call interval (64–212 vs. 49–70 ms), although the temporal ranges of calls of both species are partly overlapping. For a distinction of other new species described herein, see diagnoses in the respective species accounts below.

Description of the holotype. Adult male; specimen in good state of preservation, on the left thigh a tissue was taken for DNA analyses. SVL 25.8 mm. Body moderately slender, ratio of head length to head width HL/HW = 1.10, head slightly narrower than body, snout rounded in dorsal and lateral view; nostrils directed posterolaterally, protuberant, canthus rostralis moderately distinct; loreal region concave; tympanum distinct, rounded, ratio of tympanum diameter to eye diameter TD/ED = 0.62; supratympanic fold distinct and well developed; maxillary teeth present; vomerine teeth present arranged in two roundish aggregations; choanae rounded. Arms slender, distinct single subarticular tubercles; inner and outer metacarpal tubercles recognisable; fingers without webbing; relative length of fingers 1<2<4<3; finger disks strongly dilated; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches nostril when the hindlimb is adpressed along the

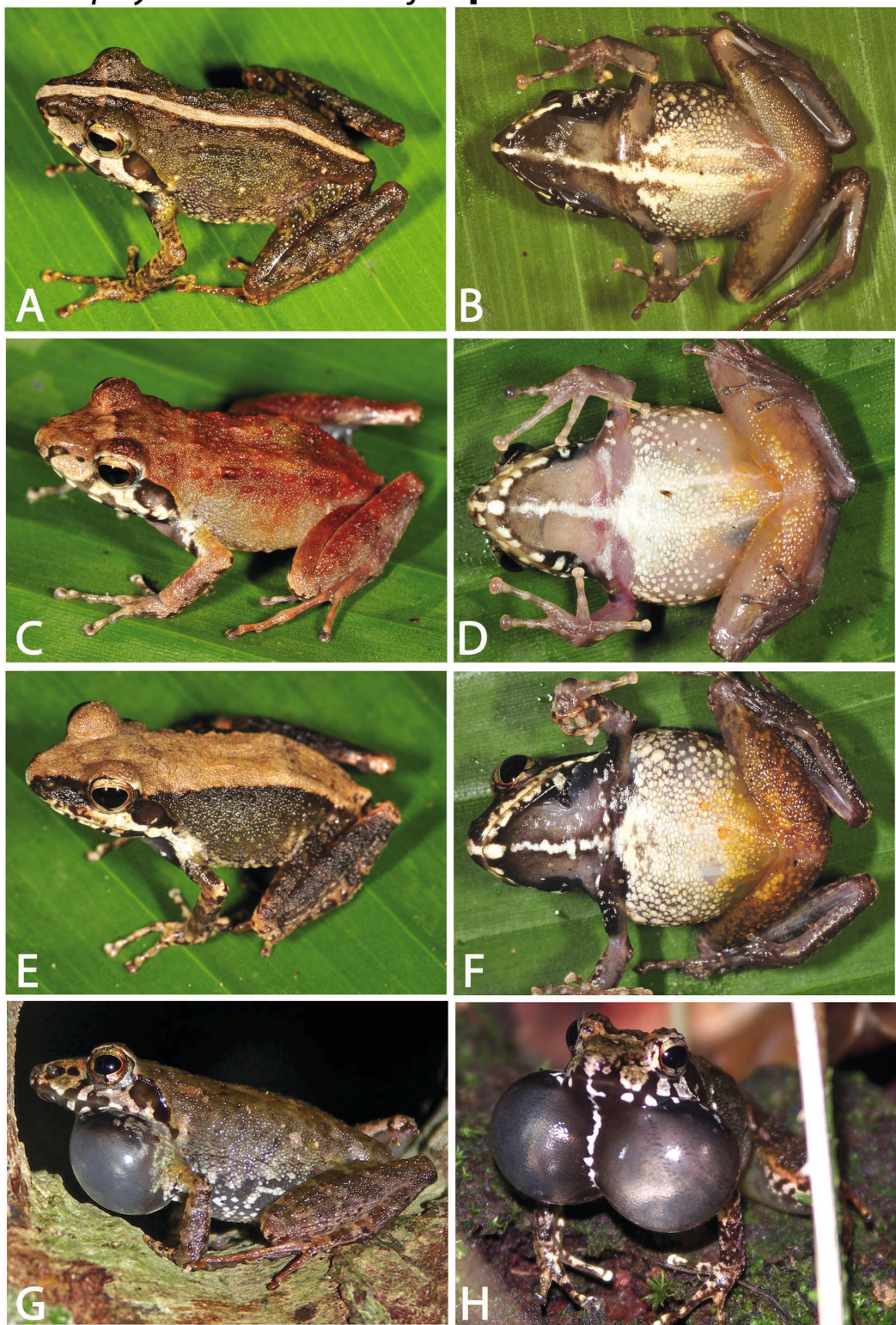# ● *Gephyromantis mitsinjo* **sp. n.**

Figure 12. Dorsal and ventral views of specimens of *Gephyromantis mitsinjo* sp. n. in life. Individuals from Anosibe An'Ala: (A, B) ZSM 304/2010, assigned by genotyping to the sublineage II; (C*, D) uncatalogued specimen; (E, F) ZSM 305/2010 (sublineage I); from Andasibe: (G) ZCMV 8132 (uncatalogued specimen, sublineage II); and from Besariaka: (H) likely ZSM 1832/2008 (ZCMV 8142), sublineage II. *Asterisk indicates mirror reversed picture.

body; lateral metatarsalia connected; inner and outer metatarsal tubercles distinct; traces of webbing between toes, more developed between toes 3, 4 and 5; relative length of toes 1<2<5<3<4. Skin on the dorsal surface almost smooth, without distinct dorsolateral ridges; flanks granular. Ventral skin smooth on throat, chest and limbs, distinctly granular on central and posterior belly. Femoral glands hardly recognisable in external view (approximately 8 poorly developed gland granules internally).

After 14 years in preservative, dorsal side of the body and limbs brown with larger slightly lighter brown markings on snout, posterior part of the head and middle of dorsum; a cream bar between the anterior part of the eyes. Dorsal surface of fore- and hindlimbs dark brown with thin lighter crossbands. Tympanum dark brown. Upper lip beige with alternating brown marbling. Vocal sacs greyish, darker along the lower lips. Throat and anterior portion of the chest brown with a white irregular median line, belly beige with brown toward the posterior portion of the chest. Lower lips dark brown with seven distinct white spots, three on each side and one on the top. Ventral surface of forelimbs with a light median zone; ventral side of thigh dirty white, including most of the femoral glands, darker laterally; ventral side of shank lighter medially (Fig. 11).

Variation. ZSM 162/2016, ZSM 0404/2006 and ZSM 5059/2005 present a lined colour morph, with a contrasting large dirty white/beige band running from snout to cloaca. ZSM 1847/2016 presents a very thin beige vertebral stripe running from snout to cloaca. ZSM 5060/2005, 5061/2005 and ZSM 159/2016 present a remarkably uniform dark brown dorsal coloration (see also Fig. 12). ZSM 162/2016 from Vohimana has an unusually small size (SVL 19.2 mm), but external sexual characters (e.g., black vocal sacs) suggest it probably being an adult male.

Etymology. We name this new species after Mitsinjo, a nongovernmental organisation in the Andasibe area, in recognition of its substantial contributions to nature conservation and amphibian conservation breeding in this part of Madagascar. The former Analamazaotra forestry station is currently managed by this NGO and often referred to as Mitsinjo forest, and the new species is common in this forest. Mitsinjo means "planning the future" in Malagasy, and the species epithet is used as a noun in apposition.

Habitat, habits, and distribution. The species is often encountered in primary as well as disturbed rainforests, as well as mature eucalypt forest with dense understory, within its distribution range. Specimens can be found on the forest floor, and males often call during the day from dense understory vegetation or from other kinds of concealed positions. Calls can also be heard at night, and specimens then sit more in the open, often perched in the low vegetation up to 1.5 m high. Calling specimens are regularly spaced across the forest and not concentrated next to water bodies.

This species is known from numerous localities in the Northern Central East: (1) the type locality, Andasibe, where the species occurs in various forest sites, including mature eucalypt forests, Analamazaotra Forestry Station, Analamazaotra-Mantadia National Park; (2) An'Ala; (3) Camp Prolemur, near Mantadia and Torotorofotsy; (4) Torotorofotsy; (5) Vohimana; (6) Maromizaha; (6) Besariaka (south of Moramanga), (7) Anosibe An'Ala, (8) Vohiposa (Anivorano Est, Andrarihitra), (9) Masobe forest for subset II, and Anosibe An'Ala as well as (10) Tarzanville for subset I (Fig. 3).

Vocalisation. Advertisement calls recorded in February 1991 at Andasibe (subset II, air temperature not taken) consist of a single pulsatile note of medium duration. Calls (= notes) are emitted in call series at rather regular intervals in fast succession. Inter-call intervals are slightly longer in duration at the beginning of a call series and get continuously shorter towards the end of the series, so call repetition within notes slightly speeds up. Structure of pulses within notes is complex with pulses being partly fused. In some notes roughly 15–27 pulses are countable. Maximum call energy is present at approximately the middle of each note. Numerical parameters of 27 analysed calls from one individual are as follows: call duration (= note duration) 78–130 ms (104.7 ± 13.1 ms); inter-call interval 64–212 ms (101.8 ± 33.0 ms); dominant frequency 4105–4359 Hz (4196 ± 99 Hz); prevalent bandwidth 3900–5200 Hz. Call series (n = 2) had a duration of 3064 and 2220 ms, and contained 15 and 12 calls, respectively. Call rate within the series varied approximately from 280–345 calls/minute (Fig. 7).

Gephyromantis kremenae sp. n.
Figs 2, 3, 4–7, 11, 13

LSID: urn:lsid:zoobank.org:act:95195CB1-2331-40AD-9F0D-429042CD1210

Remark. Referred to earlier as subset III in the present work.

Holotype. ZSM 301/2010 (FGZC 4247), adult male, from Ambodivoahangy (15.2899° S, 49.6203° E, ca. 100 m a.s.l.), northeastern Madagascar, collected on 2 April 2010 by F. Glaw, J. Köhler, P.-S. Gehring, M. Pabijan, and F. M. Ratsoavina (Figs 11, 13).

Paratypes (n = 5). ZSM 694/2009 (ZCMV 11185), ZSM 695/2009 (ZCMV 11189), both adult males from Melivinany "S OI", Manompana (no exact coordinates available), Forêt de Befanjana, Madagascar, collected on 15 May 2009 by J. E. Randrianirina; ZSM 5056/2005 (ZCMV 887), ZSM 5057/2005 (ZCMV 2121), both adult males, and ZSM 5058/2005 (ZCMV 2131), adult specimen, all from Nosy Mangabe (app. 15.5° S, 49.7° E, ca. 50 to 100 m a.s.l.), Madagascar, collected on 22 February 2005 by F. Glaw, M. Vences, and R. D. Randrianiana; ZSM 269/2016

(FGZC 5306), adult male, from Masoala, Ecolodge "Chez Arol" (app. 15.7122° S, 49.9639° E, 21 m a.s.l.), Madagascar, collected on 9 August 2016 by F. Glaw, D. Prötzel, J. Forster, K. Glaw, and T. Glaw.

Diagnosis. A member of the subgenus *Gephyromantis* in the genus *Gephyromantis* on the basis of (1) presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), (2) small size (SVL < 35 mm), (3) slightly enlarged terminal discs of fingers, (4) presence of outer metatarsal tubercle, (5) absence of webbing on hands and presence of only rudimentary webbing on feet, (6) tight connection of tissue surrounding the two lateral metatarsalia, (7) presence of femoral glands in males, (8) presence of paired/bilobed blackish vocal sacs in males, (9) diurnal calling behaviour not concentrated at water bodies, (10) molecular phylogenetic relationships.

Distinguished from all nominal species within the subgenus by combination of (1) moderate size compared to other species (male SVL up to at least 28.5 mm), (2) dorsum with coarse granules, (3) upper lip typically with alternating white/dark brown markings, (4) lower lip ventrally without a yellowish tint in life; (5) no reddish tint on ventral side of thighs in life, (6) relatively long hindlimbs (TIBL/SVL 0.50–0.58), (7) fast call series of about 9–10 calls of 86–112 ms duration, with a high call repetition rate of about 390 calls/minute. The new species is furthermore differentiated from all nominal species of *Gephyromantis,* except *G. boulengeri,* by a substantial molecular differentiation, with uncorrected pairwise distances >3% in the mitochondrial 16S gene.

The species is most similar to *G. boulengeri* and *G. mitsinjo,* both of which are also characterised by the presence of coarse granules on the dorsum and similar body size. However, *G. kremenae* is distinguished from *G. boulengeri* by its higher note repetition rate (390 vs. 116–235 calls/min), longer call duration (about 86–112 vs. 28–54 ms) and shorter inter-call intervals (49–70 vs. 217–464 ms) in advertisement calls. It is distinguished from *G. mitsinjo* by probably a higher call repetition rate (390 vs. 280–345 calls/min) and smaller number of calls in a call series (9–10 vs. 12–15 calls/series). Within the *G. boulengeri* complex, this species also differs from *G. sergei* sp. n. (described below) by shorter inter-call intervals (49–70 vs. 106–196 ms). For a distinction of other new species described herein, see diagnoses in the respective species accounts below.

Description of the holotype. Adult male; specimen in good state of preservation, but tongue taken for DNA analyses. SVL 26.5 mm. Body moderately slender, ratio of head length to head width HL/HW = 1.13, head slightly narrower than body, snout rounded in dorsal and lateral view; nostrils directed posterolaterally, protuberant, canthus rostralis moderately distinct; loreal region concave; tympanum distinct, rounded, ratio of tympanum diameter to eye diameter TD/ED = 0.63; supratympanic fold distinct and well developed; maxillary teeth present; vomerine teeth

present in two roundish aggregations; choanae rounded. Arms slender, distinct single subarticular tubercles; inner and outer metacarpal tubercles recognisable; fingers without webbing; relative length of fingers 1<2=4<3; finger disks strongly dilated; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches between eyes and nostril when the hindlimb is adpressed along the body; lateral metatarsalia connected; inner and outer metatarsal tubercles distinct; traces of webbing between toes, more developed between toes 4 and 5; relative length of toes 1<2<3=5<4. Skin on the dorsal surface granular, with distinctly elongated tubercles scattered along the back on the flanks, without distinct dorsolateral ridges; flanks with distinct round and elongated tubercules. Ventral skin smooth on throat, chest and limbs, granular on central and posterior belly. Femoral glands clearly recognisable in external view, consisting of approximately 52 small gland granules internally on the left thigh.

After 12 years in preservative, dorsal side of the body and limbs dark brown with larger slightly lighter brown markings on the head and posterior back; a dark brown bar between the eyes. Dorsal surface of fore and hindlimbs with dark brown crossbands. Tympanum dark brown. Upper lip brown with alternating white spots. Vocal sacs uniformly black. Throat and anterior portion of the chest brown with a white irregular median line, belly dirty white anteriorly with brown toward the posterior portion of the chest. Lower lips dark brown with two alternating white spots on each side. Ventral surface of forelimbs with a light median zone; ventral side of thigh dirty white, including most of the femoral glands, bordered by brown laterally; ventral side of shank with a well-defined light median zone (Figs 11, 13).

Variation. In comparison with the holotype, paratypes from Nosy Mangabe (ZSM 5056/2005 to 5058/2005) tend to have a lighter and contrasted dorsal coloration, ventral colour patterns very similar to the holotype. Paratypes from Melivinany (ZSM 694/2009, ZSM 695/2009) are slightly smaller, with a more granular skin texture (see also Fig. 13)

Etymology. We dedicate this species to Claire Kremen, University of British Columbia, in recognition of her contributions to conservation planning in Madagascar, which included pioneering studies to help setting up Masoala National Park where this species occurs.

Habitat, habits, and distribution. Similar to other species of the *G. boulengeri* complex. This predominantly diurnal species at Nosy Mangabe is often found during the day on the leaf litter of primary rainforest; the diurnal calls are heard throughout the forest, not concentrated at water bodies. This species is the representative of the subgenus *Gephyromantis* occurring furthest northwards, and is common in lowland forests at the border of the Northern Central East towards the North East region. It is known from (1) Ambodiriana, (2) Befanjana forest, (3) Ambodivoahangy, (4) Nosy Mangabe, and (5) Masoala (Fig. 3).
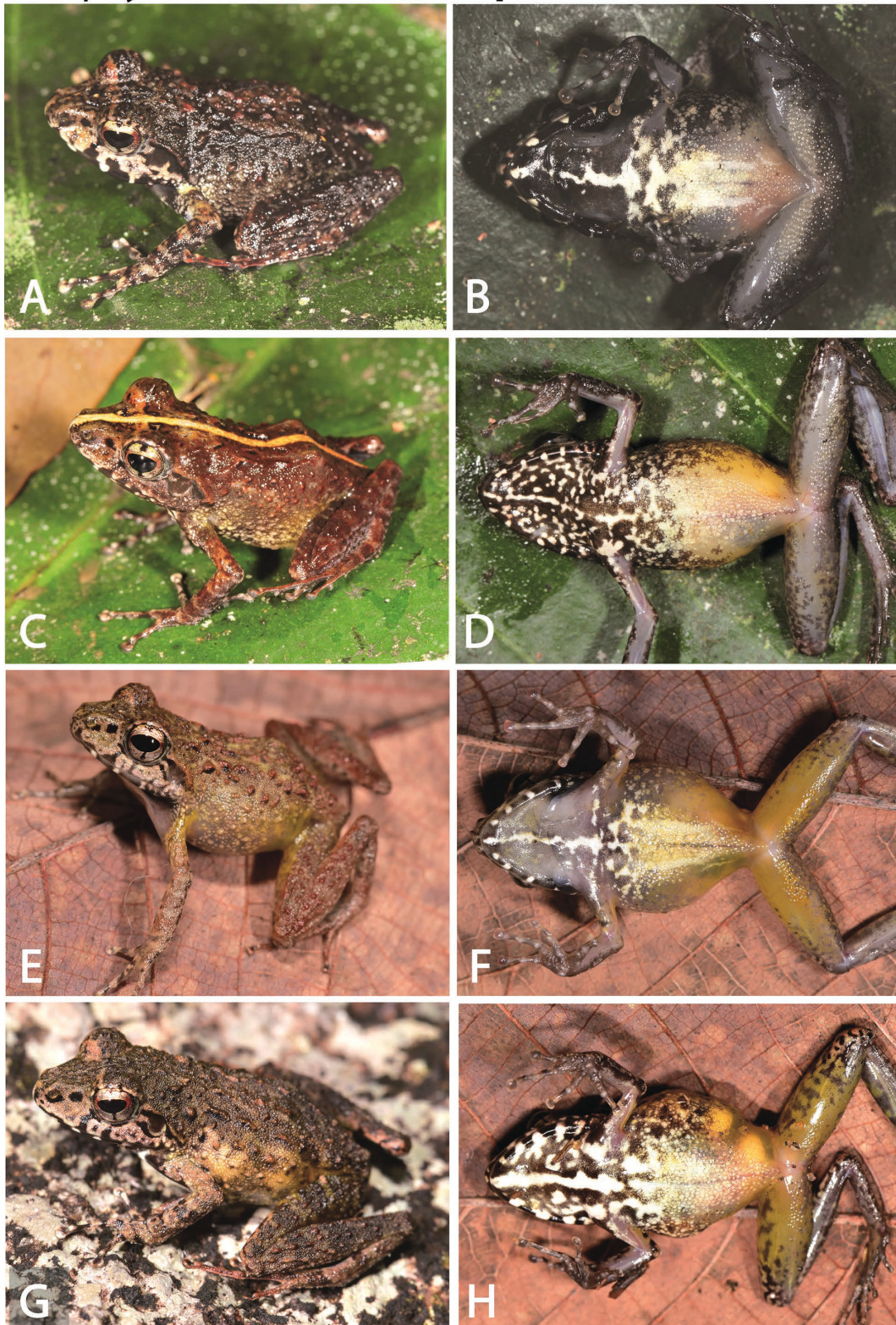
● *Gephyromantis kremenae* sp. n.

Figure 13. Dorsal and ventral views of specimens of *Gephyromantis kremenae* sp. n. in life. Individuals from Ambodivoahangy: (A*, B) ZSM 301/2010 (holotype); (C*, D) uncatalogued specimen; and from Masoala: (E* F) ZSM 269/2016; (G, H) uncatalogued specimen. *Asterisks indicate mirror reversed pictures.

Vocalisation. Advertisement calls recorded in mid-March 1991 at Nosy Mangabe (air temperature not recorded) consist of a single pulsatile note of medium duration. Calls (= notes) are emitted in call series at rather regular intervals in fast succession (Fig. 7). Structure of pulses within notes is complex and shows some variation among notes, but usually 2–4 pulses are fused to short pulse groups. Numerical parameters of 9 analysed calls from one individual are as follows: call duration (= note duration) 86–112 ms (101.7 ± 8.2 ms); inter-call interval 49–70 ms (61.1 ± 7.8 ms); dominant frequency 3177–3325 Hz (3222 ± 55 Hz); prevalent bandwidth 3000–4200 Hz. Call series (n = 1) had a duration of 1387 ms and contained 9 calls. Call rate within the series was approximately 360 calls/minute.

Two call series recorded on 2 April 2010 in the daytime at Ambodivoahangy (call voucher FGZC 4247) had durations of 1403 and 1260 ms, and contained 10 and 9 calls, respectively, repeated at a rate of approximately 390 calls/minute. Recording quality is very poor and further measurements barely possible, but call duration seems to range around 100 ms, thus being in agreement with the calls described from Nosy Mangabe.

Gephyromantis sergei sp. n.
Figs 2, 3, 4–7, 14, 15

LSID: urn:lsid:zoobank.org:act:E4A8631D-A561-4DEC-B198-97DE9348CDDC

Remark. Referred to earlier as subsets IV and V in the present work.

Holotype. ZSM 664/2003 (FGMV 2002.0243), adult male, from Ranomafana National Park (ca. 21.1550° S, 47.4994° E, ca. 1000 m a.s.l.), eastern Madagascar, collected on 18 January 2003 by F. Glaw, M. Puente, L. Raharivololoniaina, M. Thomas, and D. R. Vieites. Genotyped as belonging to the subset V (Figs 14, 15).

Paratypes (n = 14, all assigned to the subset V). ZSM 1834/2008 (ZCMV 8165), adult female, ZSM 1835/2008 (ZCMV 8168), adult male, ZSM 1836/2008 (ZCMV 8169), adult male, ZSM 1837/2008 (ZCMV 8191), adult female, ZSM 1838/2008 (ZCMV 8192), adult male, ZSM 1839/2008 (ZCMV 8170), adult female, ZSM 1840/2008 (ZCMV 8171), adult male, ZSM 1841/2008 (ZCMV 8172), adult male, ZSM 1842/2008 (ZCMV 8173), adult male, all from Talatakely (21.2567°S, 47.4236°E, 950 m a.s.l.), Ranomafana, Madagascar, collected from 13 to 16 February 2008 by K. C. Wollenberg; ZSM 2477/2007 (ZCMV 5578), ZSM 2478/2007 (ZCMV 5579), both adult males, ZSM 2479/2007 (ZCMV 5349), adult female, ZSM 2480/2007 (ZCMV 5354), adult male, ZSM 2481/2007 (ZCMV 5588), adult male, all from Ambatolahy I+II (21.2485° S, 47.4273° E, 905 m a.s.l.), Ranomafana, Madagascar, collected between 21 February and 2 March 2007 by K. C. Wollenberg, E. Rajeriarison, and T. Rajoafiarison; ZSM 2482/2007 (ZCMV

5336), adult male, from Station Thermale de Ranomafana (21.2519° S, 47.4517° E, 870 m a.s.l.), Madagascar, collected in February/March 2007 by K. C. Wollenberg, E. Rajeriarison, and T. Rajoafiarison.

Additional material (all assigned to subset IV). ZSM 2473/2007 (ZCMV 5237) unsexed adult, ZSM 2474/2007 (ZCMV 5364) adult male, ZSM 2475/2007 (ZCMV 5363), adult female, all from Ambohitsara, Tsitola forest, east of Ranomafana, Madagascar, collected in February/March 2007 by K. C. Wollenberg, E. Rajeriarison, and T. Rajoafiarison; ZSM 2476/2007 (ZCMV 5279), adult female, from the road near Ifanadiana, Madagascar, collected in February/March 2007 by K. C. Wollenberg, E. Rajeriarison, and T. Rajoafiarison; ZSM 2483/2007 (ZCMV 5876), ZSM 2484/2007 (ZCMV 5878), ZSM 2485/2007 (ZCMV 5880), ZSM 2486/2007 (ZCMV 5884), ZSM 2487/2007 (ZCMV 5886), all five adult males, from Ambohitsara, east of Ranomafana (21.3572° S, 47.8157° E, 293 m a.s.l.), Madagascar, collected on 3 March 2007 by M. Vences, K. C. Wollenberg, and E. Rajeriarison; ZSM 1843/2008 (ZCMV 8197), adult male, ZSM 1844/2008 (ZCMV 11001), adult male , ZSM 1845/2008 (ZCMV 11002), adult female, from Ranomafana, Imaloka, Madagascar, collected on 11/12 February 2008 by K. C. Wollenberg;

Gephyromantis sergei **sp. n.**
Holotype ZSM 664/2003

Gephyromantis mafifeo **sp. n.**
Holotype ZSM 509/2005

Figure 14. Dorsal and ventral views of preserved holotypes of (A) *Gephyromantis sergei* sp. n. (ZSM 664/2003) and (B) *G. mafifeo* sp. n. (ZSM 509/2005).

ZSM 634/2003 (FGMV 2002.0097), unsexed adult from Ambohitsara, east of Ranomafana (21.3572° S, 47.8157° E, 293 m a.s.l.), Madagascar, collected on 24 January 2003 by F. Glaw, M. Puente, L. Raharivololoniaina, M. Thomas, and D. R. Vieites.

Diagnosis. A member of the subgenus *Gephyromantis* in the genus *Gephyromantis* on the basis of (1) presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), (2) small size (SVL < 35 mm), (3) slightly enlarged terminal discs of fingers, (4) presence of outer metatarsal tubercle, (5) absence of webbing on hands and presence of only rudimentary webbing on feet, (6) tight connection of tissue surrounding the two lateral metatarsalia, (7) presence of femoral glands in males, (8) presence of paired/bilobed blackish vocal sacs in males, (9) diurnal calling behaviour not concentrated at water bodies, (10) molecular phylogenetic relationships.

Distinguished from all nominal species within the subgenus by the combination of (1) moderate size compared to other species (male SVL up to at least 28 mm; up to 29 mm in sublineage IV), (2) dorsum with coarse granules, (3) upper lip typically with alternating white/dark brown markings, (4) lower lip ventrally without a yellowish tint in life; (5) no reddish tint on ventral side of thighs in life, (6) relatively short hindlimbs (TIBL/SVL 0.50–0.53), (7) fast call series of about 7–12 calls of 44–112 ms duration, with a high call repetition rate of about 240–350 calls/minute. The new species is furthermore differentiated from all nominal species of *Gephyromantis* by a substantial molecular differentiation, with uncorrected pairwise distances > 3% in the mitochondrial 16S gene.

The species is most similar to *G. boulengeri*, *G. kremenae* and *G. mitsinjo*, all of which are also characterised by the presence of coarse granules on the dorsum and similar body size. However, *G. sergei* is distinguished from *G. boulengeri* by its higher note repetition rate (240–350 vs. 116–235 calls/min) and mostly a longer call duration (about 44–112 vs. 28–54 ms) and shorter inter-call intervals (106–196 vs. 217–464 ms) in advertisement calls. A fully reliable morphological distinction from *G. kremenae* and *G. mitsinjo* does not seem to be possible but based on currently available data *G. sergei* differs from *G. kremenae* by longer inter-call intervals (106–196 vs. 49–70 ms) and possibly a lower call repetition rate (240–350 vs. 390 calls/min), and from *G. mitsinjo* by a smaller number of calls in a call series (7–12 vs. 12–15 calls/series). For a distinction of other new species described herein, see diagnoses in the respective species accounts below.

Description of the holotype. Adult male; specimen in a rather good state of preservation, but the right arm was taken for DNA analyses and there is a cut in the right vocal sac. SVL 28.1 mm. Body slender, ratio of head length to head width HL/HW = 1.14, head slightly wider than body, snout rounded in dorsal and lateral view; nostrils directed posterolaterally, protuberant, canthus rostralis distinct; loreal region concave; tympanum distinct, rounded, ratio of tympanum diameter to eye diameter TD/ED = 0.63; supratympanic fold poorly developed; tongue ovoid, posteriorly bifid; maxillary teeth present; vomerine teeth present in two roundish aggregations; choanae rounded. Arms slender, distinct single subarticular tubercles; inner and outer metacarpal tubercles recognisable; fingers without webbing; relative length of fingers 1=2<4<3; finger disks distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches nostril when the hindlimb is adpressed along the body; lateral metatarsalia connected; inner and outer metatarsal tubercles distinct; traces of webbing between toes, more developed between toes 4 and 5; relative length of toes 1<2<5<3<4. Skin on the dorsal surface granular, with rather elongated tubercles scattered along the back, without distinct dorsolateral ridges; flanks with small rounded tubercules. Ventral skin smooth on throat, chest and limbs, granular on central and posterior belly. Large femoral glands clearly recognisable in external view, consisting internally of 27 gland granules on the left thigh.

After 19 years in preservative, dorsal side of the head, body and limbs almost uniformly brown. Brown cross-bands on the limbs. Laterally brown, with small whitish dots; Upper lip dirty white with brown marbling. Vocal sacs dark grey. Chest light brown with dirty white reticulations, belly dirty white. Throat brown, with a narrow broken median line made of few contrasting whitish dots; lower lips brown, with several contrasting whitish dots. Ventral surface of forelimbs with a light median zone; ventral side of thigh beige, darker laterally; ventral side of shank slightly lighter medially (Figs 14, 15).

Variation. All paratypes have a ventral colour patterns very similar to the holotype. A few specimens from Ambatolahy (e.g. ZSM 2478/2007, ZSM 2479/2007) have dorsal tubercules lighter in colour, and contrastingly bordered by dark brown (see also Fig. 15).

Etymology. The species is named after Serge H. Ndriantsoa, in recognition of his scientific contributions to our understanding of the amphibian diversity of the Ranomafana region, and his work for amphibian conservation.

Habitat, habits, and distribution. Like other species of the *G. boulengeri* complex, this species inhabits rainforest and rainforest edges, calling during the day from dense understory vegetation. Based on molecular data, the species is known from various sites in or near Ranomafana National Park. Mitochondrial sequences assigned to subset V are known from the sites Ambatolahy, Ambolo, Ataramanavana, Antenna, Station Thermale, and Talatakely, all in or directly adjacent to (1) Ranomafana National Park. Sequences assigned to subset IV are known from Ranomafana as well (sites Andalangina, Ankasaopasina, Sahalavakely, Bevoahazo, Imaloka), but also from (2) Ambohitsara and (3) a site along the road from Ifanadiana to Tolongoina (near Ifanadiana) (Fig. 3).

Vocalisation. Advertisement calls recorded on 12 March 2007 at Ranomafana, Station Thermale (subset V, air temperature unknown), consist of a single pulsatile note of medium duration. Calls (= notes) are emitted in short call series at rather regular intervals in fast succession (Fig. 7). Structure of pulses within notes is complex with pulses partly fused and barely countable. Maximum call energy is present at approximately one third of the note's duration. Numerical parameters of 12 analysed calls from one individual are as follows: call duration (= note duration) 74–96 ms (85.0 ± 7.7 ms); inter-call interval 163–196 ms (173.7 ± 11.3 ms); dominant frequency 3509–3940 Hz (3690 ± 205 Hz); prevalent bandwidth 3300–4500 Hz. Call series (n = 1) had a duration of 3196 ms and contained 12 calls. Call rate within the series was approximately 240 calls/minute.

Advertisement calls recorded at Ambohitsara in 2007 (subset IV, air temperature not recorded, but likely around 26°C) consist of a single pulsatile note of medium duration. Calls (= notes) are emitted in short call series at rather reg-

ular intervals in fast succession (Fig. 7). Structure of pulses within notes is complex with pulses partly fused and barely countable. Maximum call energy is present at the beginning of the note, constantly decreasing towards its end. Numerical parameters of 7 analysed calls from one individual are as follows: call duration (= note duration) 90–112 ms (96.1 ± 8.7 ms); inter-call interval 107–136 ms (117.0 ± 10.4 ms); dominant frequency 3620–4134 Hz (3881 ± 195 Hz); prevalent bandwidth 3500–4400 Hz. Call series (n = 1) had a duration of 1386 ms and contained 7 calls. Call rate within the series was approximately 280 calls/minute.

One call series recorded on 7 March 2007 at Ifanadiana generally agrees call parameters with that described from Ambohitsara (subset IV), except for call duration being slightly shorter and notes being barely pulsatile, almost tonal in character. Numerical parameters of 7 analysed calls from one individual are as follows: call duration (= note duration) 44–56 ms (47.0 ± 2.9 ms); inter-call interval 106–133 ms (125.0 ± 10.2 ms); dominant frequency
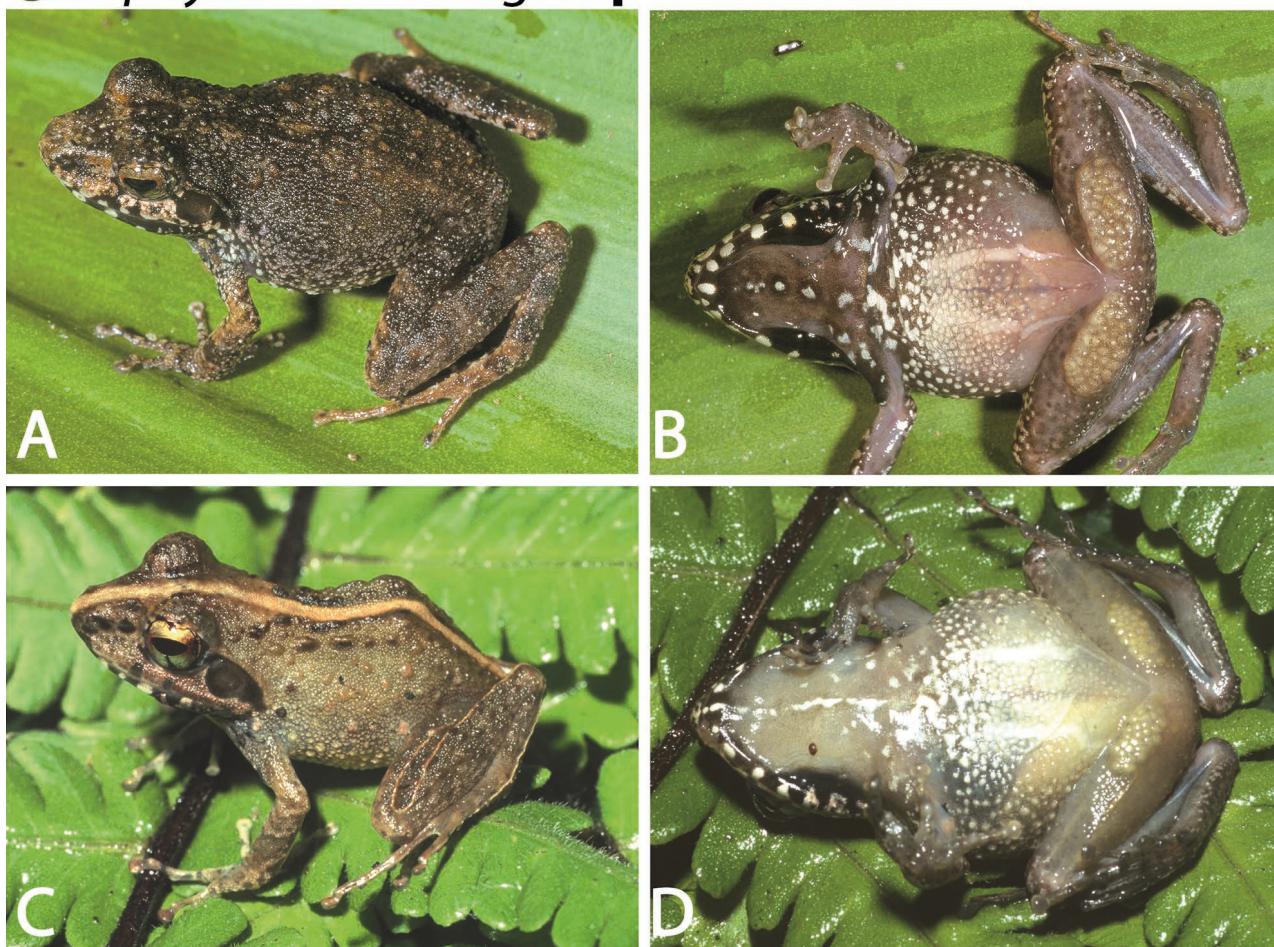
Gephyromantis sergei sp. n.

Figure 15. Dorsal and ventral views of specimens of *Gephyromantis sergei* sp. n. in life. Individuals from Ranomafana: (A, B) ZSM 664/2003 (holotype, assigned by genotyping to the sublineage V); and from Ambohitsara: (C, D), specimen tentatively assigned to the sublineage IV.

3640–3741 Hz (3702 ± 44 Hz); prevalent bandwidth 3500–4800 Hz. The call series had a duration of 1109 ms and consisted of 7 calls. Call rate within the series was approximately 350 calls/minute.

Gephyromantis mafifeo sp. n.
Figs 2, 3, 4–6, 14

LSID: urn:lsid:zoobank.org:act:71B0B739-6E07-40C5-824E-12AD547986C2

Remark. Referred to earlier as subset VI in the present work.

Holotype. ZSM 509/2009 (ZCMV 8993), adult male, from a site between Ambinanindrano and Mahanoro (19.9600° S, 48.6769° E, 51 m a.s.l.), eastern Madagascar, collected in April 2009 by F. M. Ratsoavina, E. Rajeriariason, and F. Randrianasolo (Fig. 14).

Paratypes (n = 3). ZSM 506/2009 (ZCMV 8990), ZSM 507/2009 (ZCMV 8991), ZSM 508/2009 (ZCMV 8992), all adult males, same locality, collectors and collecting date as holotype.

Diagnosis. A member of the subgenus *Gephyromantis* in the genus *Gephyromantis* on the basis of (1) presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), (2) small size (SVL < 35 mm), (3) slightly enlarged terminal discs of fingers, (4) presence of outer metatarsal tubercle, (5) absence of webbing on hands and presence of only rudimentary webbing on feet, (6) tight connection of tissue surrounding the two lateral metatarsalia, (7) presence of femoral glands in males, (8) presence of paired/bilobed blackish vocal sacs in males, (9) diurnal calling behaviour not concentrated at water bodies, (10) molecular phylogenetic relationships.

Distinguished from all nominal species within the subgenus by combination of (1) male SVL 24–26 mm, (2) dorsum with coarse granules, (3) upper lip typically with alternating white/dark brown markings, (4) lower lip ventrally without a yellowish tint in life; (5) no reddish tint on ventral side of thighs in life, (6) relatively short hindlimbs (ratio TIBL/SVL 0.50–0.53). The new species is furthermore differentiated from all nominal species of *Gephyromantis* by a substantial molecular differentiation, with uncorrected pairwise distances > 3% in the mitochondrial 16S gene.

A reliable non-molecular diagnosis of this species within the *G. boulengeri* complex is difficult due to the lack of bioacoustic data. However, the species clearly forms a clade with the two sublineages of *G. sergei*, and differs from this species by a consistently smaller body size (male SVL 24–26 vs. 26–29 mm). Also *G. kremenae* and *G. mitsinjo* are known to attain larger sizes (up to 28.5 mm male SVL). A morphological distinction of the new species from *G. boulengeri* does not seem to be possible based on the available data, but the two forms are phylogenetically not each oth-

er's closest relatives and differ in numerous diagnostic sites in the 16S rRNA gene.

Description of the holotype. Adult male; specimen in good state of preservation, on the left thigh a tissue was taken for DNA analyses. SVL 26.2 mm. Body moderately slender, ratio of head length to head width HL/HW = 1.19, head slightly narrower than body, snout rounded in dorsal and lateral view; nostrils directed posterolaterally, protuberant, canthus rostralis moderately distinct; loreal region concave; tympanum distinct, rounded, ratio of tympanum diameter to eye diameter TD/ED = 0.71; supratympanic fold distinct and moderately developed; maxillary teeth present; vomerine teeth present in two roundish aggregations; choanae rounded. Arms slender, distinct single subarticular tubercles; inner and outer metacarpal tubercles recognisable; fingers without webbing; relative length of fingers 1≤2<4<3; finger disks strongly dilated; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches nostrils when the hindlimb is adpressed along the body; lateral metatarsalia connected; inner and outer metatarsal tubercles distinct; weak traces of webbing between toes 4 and 5; relative length of toes 1<2<3=5<4. Skin on the dorsal surface finely granular, with weakly expressed elongated tubercules, without distinct dorsolateral ridge on the anterior part of the body; flanks granular. Ventral skin smooth on throat, chest and limbs, distinctly granular on central and posterior belly. Femoral glands well recognisable in external view (approximately 49 gland granules internally).

After 15 years in preservative, dorsal side of the body and limbs brown with larger slightly lighter brown markings on posterior part of the head and middle of dorsum; a light bar between the eyes. Dorsal surface of fore and hindlimbs light brown with darker crossbands. Tympanum dark brown, with dark brown marks posteriorly. Upper lip beige to light brown with few dark brown dots. Vocal sacs greyish with white spots. Throat and anterior portion of the chest dark beige/brown with a whitish reticulation forming an irregular median line, belly whitish. Lower lips discontinuously dirty white with brown interruptions. Ventral surface of forelimbs with a light median zone; ventral side of thigh dirty beige, including most of the femoral glands, darker laterally; ventral side of shank lighter medially (Fig. 14).

Variation. All paratypes have dorsal and ventral colour patterns roughly similar to that of the holotype, except for the specimen ZSM 506/2009 which presents a very distinctive light vertebral line running from the snout to the cloaca. Few specimens from Ambatohaly (ex. ZSM 2478/2007, 2479/2007) present dorsal tubercles lighter in colour, and contrastingly bordered by dark brown.

Etymology. The species epithet is a noun in apposition, derived from the Malagasy words mafy = loud, and feo = sound, with the 'y' in mafy becoming an 'i' in composite Malagasy words. The name refers to the loud and conspicuous call of this species that was noted at the time of collection.

Habitat, habits and distribution. The species is so far only known from its type locality, located along Madagascar's east coast between Ambinanindrano and Mahanoro

Vocalisation. Unknown, not recorded.

Gephyromantis blanci species complex

Gephyromantis blanci Guibé, 1973
Figs. 2–6, 8, 9, 16

Remarks. Referred to earlier as subset "blanci" in the present work.

Holotype. MNHN 1972.0183. Type locality "Mare temporaire en forêt d'Ambalamarovandana (1500 m)" [Temporary pond in the forest of Ambalamarovandana, 1500 m], Andringitra Massif, Madagascar, collected on 17 April 1971 by C.-P. Blanc (Fig. 9).

Material examined (n = 6). ZSM 2468/2007 (ZCMV 5283), ZSM 2469/2007 (ZCMV 5285), ZSM 2470/2007 (ZCMV 5290), ZSM 2471/2007 (ZCMV 5295), all unsexed adults, collected in Andringitra (no exact locality, but probably from Imaitso forest, 22.1403° S, 46.9469° E, 1509 m a.s.l.), Madagascar, collected on 1 and 2 March 2007 by E. Rajeriarison; ZSM 973/2003 (FGMV 2002.2178), adult male, from Andringitra, forest near a stream (22.1309° S, 46.8924° E, 1565 m a.s.l.), Madagascar, collected on 11 February 2003 by G. Aprea, M. Puente, L. Raharivololoniaina, M. Thomas, and D. R. Vieites; ZSM 823/2014 (ZCMV 14756), unsexed adult, Pic d'Ivohibe Special Reserve, Camp 3 (22.4967° S, 46.9564° E, 1487 m a.s.l.), Madagascar, collected on 10 November 2014 by A. Rakotoarison, M. Bletz, D. Edmonds, and F. Randrianasolo.

Diagnosis. A member of the subgenus *Gephyromantis* in the genus *Gephyromantis* on the basis of (1) presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), (2) small size (SVL < 35 mm), (3) slightly enlarged terminal discs of fingers, (4) presence of outer metatarsal tubercle, (5) absence of webbing on hands and presence of only rudimentary webbing on feet, (6) tight connection of tissue surrounding the two lateral metatarsalia, (7) presence of femoral glands in males, (8) presence of paired/bilobed blackish vocal sacs in males, (9) diurnal calling behaviour not concentrated at water bodies, (10) molecular phylogenetic relationships.

Distinguished from all nominal species within the subgenus by combination of (1) moderate size compared to other species (male SVL up to at 25.0 mm), (2) dorsum smooth, with distinct dorsolateral ridges, (3) upper lip typically uniformly light, without a distinct pattern of alternating white/dark brown markings, (4) lower lip ventrally without or with only weakly expressed yellowish tint in life; (5) no distinct reddish tint on ventral side of thighs in life, (6) short

series always containing 5 calls only, distinguishing it from all other species. This species is furthermore differentiated from all nominal species of *Gephyromantis,* except *G. enki,* by a substantial molecular differentiation, with uncorrected pairwise distances > 3% in the mitochondrial 16S gene.

The species is most similar to *G. enki* and *G. runewsweeki,* which all three form a distinct clade. However, *G. boulengeri* is distinguished from *G. enki* by a larger size (male SVL around 22.4–25.0 vs. 18.7–21.6 mm), from *G. runewsweeki* by a shorter call duration (24–33 vs. 97–212 ms) and from both species by a shorter inter-call interval (78–87 vs. 371–466 ms for *G. enki,* and 411–510 ms for *G. runewsweeki*) in advertisement calls. For a distinction of other species described herein, see diagnoses in the respective species accounts below.

Habitat, habits and distribution. At Andringitra (Imaitso forest), calling males were observed in January during the day on the bottom of (somewhat degraded) forest (Glaw & Vences 1994), not concentrated at water bodies. Based on molecular and bioacoustic data presented herein, the species is known from (1) various sites in the Andringitra Massif (type locality d'Ambalamarovandana forest; Imaitso forest; Namoly), and from (2) Pic d'Ivohibe. All sites with reliable elevational information are around 1500 m a.s.l. (Fig. 3).

Vocalisation. Advertisement calls recorded on 16 January 1994 at Imaitso forest near Ambalamarina, Andringitra (air temperature 19°C), consist of a short single note, almost tonal in character. Calls (= notes) are emitted in short call series containing 5 calls, repeated at regular intervals in rapid succession. Maximum call energy is present at the beginning of the call, rapidly decreasing towards its end. Numerical parameters of 20 analysed calls are as follows: call duration (= note duration) 24–33 ms (28.1 ± 2.6 ms); inter-call interval 78–87 ms (82.5 ± 3.5 ms); dominant frequency 3812–4080 Hz (3991 ± 162 Hz); prevalent bandwidth 3200–5000 Hz. Call series (n = 4) had a duration of 453–482 ms (470.3 ± 12.4 ms) and all contained 5 calls. Call rate within series was approximately 545 calls/minute (Fig. 8).

Gephyromantis feomborona sp. n.
Figs 2–6, 8, 16, 17

LSID: urn:lsid:zoobank.org:act:ACE19636-A662-4678-A14F-C32320D449B6

Remarks. Referred to as *G.* sp. 4 or *G.* sp. Ca4 in previous studies, and earlier as subset VII in the present work. The call described by Glaw & Vences (2000) from Vohiparara as that of *G. blanci* actually corresponds to this species.

Holotype. ZMA 20025 (ZCMV 123), adult male, from Ranomafana, Ranomafanakely (21.2487° S, 47.3718° E, 1134 m a.s.l.), eastern Madagascar, collected on 23 January 2004 by M. Vences, E. Randriamitso, D. R. Vieites, and I. De la Riva (Figs 16, 17) and seen calling by MV.

Paratypes (n = 2). ZSM 2459/2007 (ZCMV 5240), adult specimen, ZSM 2553/2007 (ZCMV 5299), adult male, from Ranomafana, Ranomafanakely (no exact coordinates of collecting site), Madagascar, collected on 5 and 2 March 2007, respectively, by K. C. Wollenberg, E. Rajeriarison, and T. Rajoafiarison.

Diagnosis. A member of the subgenus *Gephyromantis* in the genus *Gephyromantis* on the basis of (1) presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), (2) small size (SVL < 35 mm), (3) slightly enlarged terminal discs of fingers, (4) presence of outer metatarsal tubercle, (5) absence of webbing on hands and presence of only rudimentary webbing on feet, (6) tight connection of tissue surrounding the two lateral metatarsalia, (7) presence of femoral glands in males, (8) presence of paired/bilobed blackish vocal sacs in males, (9) diurnal calling behaviour not concentrated at water bodies, (10) molecular phylogenetic relationships.

Distinguished from all nominal species within the subgenus by combination of (1) small size compared to other species (male SVL 21–22 mm), (2) dorsum smooth, with weakly expressed but clearly visible outer dorsolateral ridges, (3) upper lip relatively uniform light, without strongly expressed alternating white/dark brown markings, (4) lower lip ventrally in most cases without a yellowish tint in life; (5) no reddish tint on ventral side of thighs in life; (6 relatively short hindlimbs (ratio TIBL/SVL 0.54–0.56), (7) call series consisting of about 11–14 calls of 15–29 ms duration,

● *Gephyromantis feomborona* **sp. n.**
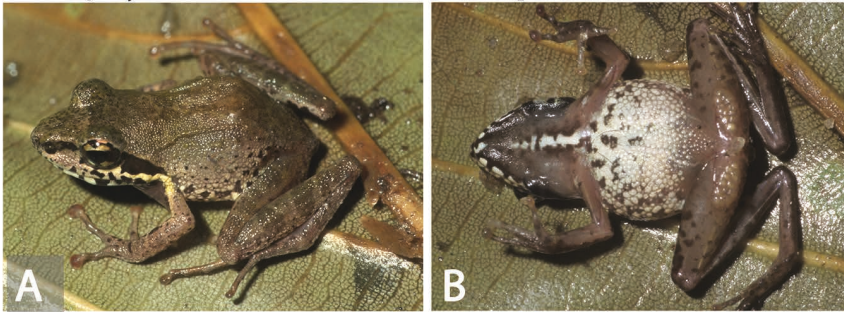
● *Gephyromantis cornucopia* **sp. n.**

● *Gephyromantis blanci*

Figure 16. Dorsal and ventral views of specimen of *Gephyromantis feomborona* sp. n. in life from (A, B) Vohiparara, ZMA 20025 (holotype); of *G. cornucopia* sp. n. in life from Andohahela; (C) ZSM 183/2005 (holotype); (D, E) two uncatalogued specimens; and of *G. blanci* in life from (F, G) Andringitra, uncatalogued specimen photographed in 1994; and from (H*) Pic d'Ivohibe, ZSM 823/2014. *Asterisk indicates mirror reversed picture.

with a high call repetition rate of about 520–660 calls/minute. The new species is furthermore differentiated from all nominal species of *Gephyromantis* by a substantial molecular differentiation, with uncorrected pairwise distances > 3% in the mitochondrial 16S gene.

The new species is morphologically most similar to *G. blanci*, *G. cornucopia* sp. n. (described below), *G. enki*, and *G. runewsweeki*. It differs from *G. blanci* by slightly smaller body size (male SVL 21–22 vs. 22–23 mm) and a higher number of calls per call series (11–14 vs. 5 calls/series); from *G. enki* by a much higher call repetition rate, a shorter call duration (15–29 vs. 31–34 ms) and by absence of yellow colour on lower lip and in frenal stripe (vs. often present); from *G. cornucopia* sp. n. by a smaller size (male SVL 21.2–22.2 vs. 25.9–27.5 mm), a shorter call duration (15–29 vs. 83–118 ms) and a longer inter-call interval (58–109 vs. 21–62 ms), and from *G. runewsweeki* by the emission of single call series after long, irregular intervals, vs. multiple call series in a row and a much shorter call duration (15–29 vs. 97–212 ms). Furthermore, it differs from *G. eiselti*, *G. mafy* and *G. thelenae* by the presence of outer dorsolateral ridges (vs. usually not recognisable in those species), by absence of reddish ventral colour (vs. present in *G. thelenae*) and usually absence of yellowish colour on lower lip (vs. presence), and by very different advertisement call (much faster call repetition rate and much shorter call duration and inter-call interval).

Description of the holotype. Adult male; specimen in good state of preservation, on the right thigh a tissue was taken for DNA analyses. SVL 21.2 mm. Body slender, ratio of head length to head width HL/HW = 1.25, head slightly wider than body, snout rounded in dorsal and lateral view; nostrils directed posterolaterally, protuberant, canthus rostralis distinct; loreal region concave; tympanum distinct, slightly oval (higher than wide), ratio of tympanum diameter to eye diameter TD/ED = 0.58; supratympanic fold distinct but weakly developed; tongue ovoid, posteriorly bifid; maxillary teeth present; vomerine teeth absent; choanae rounded. Arms slender, distinct single subarticular tubercles; inner and outer metacarpal tubercles recognisable; fingers without webbing; relative length of fingers 1≤2<4<3; finger disks distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches slightly beyond snout tip when the hindlimb is adpressed along the body; lateral metatarsalia connected; inner and outer metatarsal tubercles distinct; traces of webbing between toes; relative length of toes 1<2<5≤3<4. Skin on the dorsal surface slightly tuberculated (almost smooth), with continuous, moderately developed dorsolateral ridges in the middle section of the dorsum; skin on the lateral sides tuberculated with additional short ridges. Ventral skin smooth on throat, chest and limbs, granular on central and posterior belly. Femoral glands distinctly visible in external view, consisting of 12 large gland granules irregularly arranged in two rows in internal view (checked on right thigh only).

After 18 years in preservative, dorsum between dorsolateral ridges and snout region brown; a colour border is present between the eyes, with lighter colour anteriorly. Laterally brown, without dorsolateral colour border; a dark brown marking bordered by supratympanic fold, insertion of forelimbs and posterior edge of the eye, ventrally bordered by an irregular whitish stripe along the upper lip; upper lip and tip of snout whitish with diffuse dark mottling. Arms and legs dorsally with diffuse light and dark brown mottling and crossbands. Vocal sacs black. Chest dirty white with brown spots, belly dirty white, ventral surfaces of hindlimbs (including femoral glands) beige. Throat brown, with a narrow, partly broken, light median line; lower lips with alternating whitish and brown marbling. Ventral surfaces of forelimbs with a light median zone; ventral side of thigh white, bordered by brown colour laterally; ventral side of shank with a well-defined light median zone (Figs 16, 17).

Variation. Paratype ZSM 2459/2007 has a finely reticulated light and dark brown dorsal coloration. Its dorsal surface is very pale (dirty white) with fine sharp dark brown reticulation at a wide interval. Paratype ZSM 2553/2007 is characterised by a lined colour morph made of five dark brown stripes on a lighter background: two broad bands on the flanks, two broad paravertebral bands and a very thin discontinuous vertebral line (see also Fig. 16).

Etymology. The species epithet is composed of the Malagasy words 'feo' (voice, sound), and 'borona' (= vorona; bird), and refers to the bird-like trill call of this species. The name is a noun in apposition.

● *G. feomborona* **sp. n.**
Holotype ZMA 20025

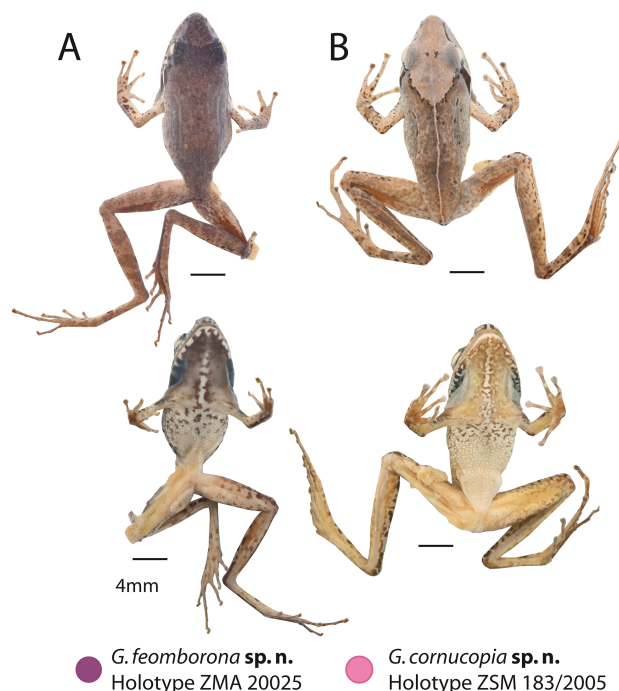● *G. cornucopia* **sp. n.**
Holotype ZSM 183/2005

Figure 17. Dorsal and ventral views of preserved holotypes of (A) *Gephyromantis feomborona* sp. n. (ZMA 20025); and (B) *G. cornucopia* sp. n. (ZSM 183/2005).

Habitat, habits, and distribution. We heard calls of this species in primary rainforest, or at rainforest edges. It can occur in close syntopy with the morphologically very similar *G. enki* which in Ranomafana appears to be more widespread judging by its almost omnipresent calls in some areas. At the main road close to Vohiparara, calls of *G. feomborona* were heard from dense understory vegetation during the day. Since the calls of this species are emitted only after longer intervals, locating calling specimens is extremely difficult. One individual was perched at a concealed position about 1 m high in the vegetation on fern fronds. The species is known to be nidicolous; the development of its endotrophic (non-feeding) tadpoles in a terrestrial jelly nest was described by Randrianiaina et al. (2011) (under the name *G.* sp. aff. *blanci*). *G. feomborona* is only known from Ranomafana National Park, where genetic and bioacoustic records are available from Ranomafanakely and Vohiparara, and probable calls have also been heard at Maharira (Fig. 3).

Vocalisation. Advertisement calls recorded on 28 February 1996 (18:10 h, air temperature unknown) at Vohiparara, consist of a short single note, almost tonal in character. Calls (= notes) are emitted in call series and repeated at rather regular intervals. Maximum call energy is present in the first half of the call, rapidly decreasing towards its end. Numerical parameters of 42 analysed calls of 3 individuals are as follows: call duration (= note duration) 15–29 ms (22.7 ± 3.7 ms); inter-call interval 58–109 ms (74.9 ± 14.6 ms); dominant frequency 4404–4555 Hz (4446 ± 55 Hz); prevalent bandwidth 3700–5300 Hz. Call series (n = 5) had a duration of 1038–1309 ms (1200.8 ± 111.0 ms) and contained 11–14 calls (12.8 ± 1.1). Call rate within series ranged approximately from 520–660 calls/minute (Fig. 8).

Gephyromantis cornucopia sp. n.
Figs 2–6, 16, 17

LSID: urn:lsid:zoobank.org:act:8CB37D3E-5ABE-43E9-9D51-D1E6AFDF530E

Remarks. Referred to as *G.* sp. 5 or Ca5 in previous studies, and earlier as subset VIII in the present work.

Holotype. ZSM 183/2005 (FGZC 2462), adult male, from Andohahela National Park (24.5440° S, 46.7141° E, 1548 m a.s.l.), Anosy region, southeastern Madagascar, collected on 27 January 2005 by F. Glaw, M. Vences, and P. Bora (Figs 16, 17).

Paratypes (n = 7). ZSM 182/2005 (FGZC 2459), subadult, ZSM 184/2005 (FGZC 2463), female, ZSM 185/2005 (FGZC 2464), female, ZSM 186/2005 (FGZC 2471), female, and ZSM 187/2005 (FGZC 2473), female, all with same data as holotype; ZSM 822/2014 (ZCMV 14727), adult male, Pic d'Ivohibe Special Reserve (22.4819° S, 46.9515° E, 971 m a.s.l.), Marovitsika, Madagascar, collected on 8 November 2014 by A. Rakotoarison, M. Bletz, D. Edmonds, and

F. Riandrianasolo; ZSM 361/2016 (ZCMV 14861), adult male, from 3 km up from Sampanandrano (Anosy Mountain Chain, southern Madagascar ; Sampanandrano coordinates being 24.1399° S, 49.0742° E, 539 m a.s.l.), collected on 17 December 2016 by A. Rakotoarison, E. Rajeriarison, and J. W. Ranaivosolo.

Additional material examined (n = 96). MNHN-RA-1972.1846, 1972.1847, 1972.1849, 1972.1850, 1972.1854, 1972.1855, 1972.1857, 1972.1859, 1972.1860, 1972.1862 to 1864, 1972.1871 to 1874, 1972.1876, 1972.1878, 1972.1880 to 1887, 1972.1889 to 1893, 1972.1897, 1972.1901, 1972.1905, 1972.1906, 1972.1908, 1972.1910 to 1920, 1972.1922, 1972.1923, 1972.1925 to 1928, 1972.1931 to 1934, 1972.1936, 1972.1939, 1972.1942 to 1944, 1972.1946 to 1948, 1972.1950, 1972.1954, 1972.1955, 1972.1956, 1972.1958, 1972.1962, 1972.1963, 1972.1967 to 1970, 1972.1972, 1972.1975 to 1977, 1972.1979, 1972.1987, 1972.1992, 1972.1993, 1972.1995 to 1997, 1972.1999, 1972.2000, 1972.2009, 1972.2013, 1972.2025, 1972.2034, 1972.2054, 1972.2063, 1972.2065, 1972.2069, all from the Anosy mountain range, "Chaînes Anosyennes, Camp 3, 4, 5", collected by C.-P. Blanc.

Diagnosis. A member of the subgenus *Gephyromantis* in the genus *Gephyromantis* on the basis of (1) presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), (2) small size (SVL < 35 mm), (3) slightly enlarged terminal disks of fingers, (4) presence of outer metatarsal tubercle, (5) absence of webbing on hands and presence of only rudimentary webbing on feet, (6) tight connection of tissue surrounding the two lateral metatarsalia, (7) presence of femoral glands in males, (8) presence of paired/bilobed blackish vocal sacs in males, (9) diurnal calling behaviour not concentrated at water bodies, (10) molecular phylogenetic relationships.

Distinguished from all nominal species within the subgenus by combination of (1) small size compared to other species (male SVL 26–27 mm), (2) dorsum smooth, with weakly expressed but clearly visible outer dorsolateral ridges, (3) upper lip relatively uniform light, without strongly expressed alternating white/dark brown markings, (4) lower lip ventrally in most cases without a yellowish tint in life; (5) no reddish tint on ventral side of thighs in life; (6) relatively long hindlimbs (ratio TIBL/SVL 0.55–0.60), (7) probably fast call series of about 17–25 calls of 21–62 ms duration, with a high call repetition rate of about 390–420 calls/minute. The new species is furthermore differentiated from all nominal species of *Gephyromantis* by a substantial molecular differentiation, with uncorrected pairwise distances > 3% in the mitochondrial 16S gene.

The new species is morphologically most similar to *G. blanci*, *G. enki*, *G. feomborona*, and *G. runewsweeki*. It differs from *G. blanci* by larger body size (male SVL 26–27 vs. 22–23 mm), a much longer call duration (83–118 vs. 24–33 ms), a shorter call interval (21–62 vs. 78–87 ms), and a higher number of calls per call series (17–25 vs. 5 calls/series); from *G. enki* by larger body size (male SVL 26–27 vs. 20–21 mm), by much higher call repetition rate, a much

longer call duration (83–118 vs. 31–34 ms), a much shorter inter-call intervals (21–62 vs. 371–466 ms) and by absence of yellow colour on lower lip and in frenal stripe (vs. often present); from *G. feomborona* by a larger size (male SVL 25.9–27.5 vs. 21.2–22.2 mm), a much longer call duration (83–118 vs. 15–29 ms) and tendency to exhibit shorter inter-call intervals (21–62 vs. 58–109 ms); and from *G. runewsweeki* by larger body size (male SVL 26–27 vs. 23–24 mm), a much shorter inter-call interval (21–62 vs. 411–510 ms) and the emission of mostly single call series after long, irregular intervals (vs. multiple call series in a row). Furthermore, it differs from *G. eiselti*, *G. mafy* and *G. thelenae* by the presence of outer dorsolateral ridges (vs. usually not recognisable in those species), by larger body size (male SVL 26–27 vs. 19–22 mm), by absence of reddish ventral colour (vs. present in *G. thelenae*) and usually absence of yellowish colour on lower lip (vs. presence), and by a very different advertisement call (much higher call repetition rate, shorter call duration and shorter inter-call interval).

Description of the holotype. Adult male; specimen in good state of preservation, on the right thigh a tissue was taken for DNA analyses. SVL 25.9 mm. Body slender, ratio of head length to head width HL/HW = 1.20, head slightly wider than body, snout rounded in dorsal and lateral view; nostrils directed posterolaterally, protuberant, canthus rostralis distinct; loreal region concave; tympanum distinct, rounded (slightly flatten dorsally by the supratympanic fold), ratio of tympanum diameter to eye diameter TD/ED = 0.56; supratympanic fold distinct but weakly developed; tongue ovoid, posteriorly bifid; maxillary teeth present; vomerine teeth absent; choanae rounded. Arms slender, distinct single subarticular tubercles; inner and outer metacarpal tubercles recognisable; fingers without webbing; relative length of fingers 1<2=4<3; finger disks distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches slightly beyond snout tip when the hindlimb is adpressed along the body; lateral metatarsalia connected; inner and outer metatarsal tubercles distinct; traces of webbing between toes, more developed between toes 4 and 5; relative length of toes 1<2<5=3<4. Skin on the dorsal surface almost smooth, with continuous, distinctly developed dorsolateral ridges in the middle section of the dorsum; skin on the lateral sides tuberculated without additional ridges. Ventral skin smooth on throat, chest and limbs, granular on central and posterior belly. No recognisable femoral glands, neither internally nor externally (femoral gland destroyed by tissue sampling on the right thight, not recognisable on the left thigh).

After 17 years in preservative, dorsal side of the body and limbs brown, with thin dark dots on dorsum and indistinct brown crossbands on the limbs. Dorsal side of the head beige, strongly contrasting with the darker dorsum. A thin light vertebral line runs from the posterior part of the head to the vent. Two small dark spots between the eyes. Laterally brown, slightly lighter than dorsally; a dark irregular narrow band between the nostril and the anterior edge of the eye, continuing posteriorly to the eye up to the insertion of forelimb, and dorsally bordered by the supratympanic fold. Upper lip dirty white with few fine dark dots. Vocal sacs greyish, with dark reticulations laterally. Chest dirty white with small brown spots, belly dirty white. Throat beige, with a narrow median light line; lower lips with very diffuse alternating of whitish and brown dots. Ventral surface of forelimbs with a light median zone; ventral side of thigh yellowish, bordered by brown colour laterally; ventral side of shank with a well-defined light median zone (Figs 16, 17).

Variation. Paratype ZSM 187/2015 (from the type locality) has a uniform dark brown dorsal surface with a contrasting large dirty white band running from snout to cloaca. Dark brown crossbands on hindlimbs. Paratypes ZSM 184/2005 (from the type locality) and ZSM 822/2014 (from Pic d'Ivohibe) present both a pale beige uniform dorsal coloration, with light brown crossbands on hindlimbs. All the paratypes have a sharp coloration border between a light snout and a darker posterior part of the head (see also Fig. 16). Contrary to the holotype, femoral glands are well recognisable in ZSM 822/2014 and ZSM 361/2016.

Etymology. In classical antiquity, the cornucopia, from Latin 'cornu' (horn) and 'copia' (abundance), also called the horn of plenty, was a symbol of abundance. This specific epithet, used as an invariable noun in apposition, refers to the seemingly never-ending species inventory of life on Earth, a trend well exemplified by Malagasy amphibians.

Habitat, habits and distribution. Very poorly known. At Andohahela, we heard calls probably assignable to this species (see below) during the day from primary rainforest, not concentrated near water bodies. Specimens were found on the forest floor. The species is known from (1) the type locality, Andohahela National Park, (2) Pic d'Ivohibe Special Reserve, and (3) the Anosy Mountain chain, based on a genotyped specimen from near Sampanandrano. A large number of specimens from the Anosy Mountain Chain preserved in the MNHN may also belong to this species, but the identity of these specimens could not be verified with full reliability (Fig. 3).

Vocalisation. Advertisement calls recorded on 27 January 2005 at Andohahela (air temperature 16.9°C) are here tentatively considered to represent calls of *G. cornucopia*, because this was the only species of *Gephyromantis* found in the respective forest patches. Recording quality is rather poor and suffers from loud background noise, partly hampering proper analysis. Therefore, values and characters provided have to be taken with some reservation. Calls consist of a pulsatile single note of medium duration, repeated at very fast succession in call series. Pulses within notes are apparently numerous but show a complex structure and are partially fused. Maximum call energy is present at the beginning of the call, constantly decreasing towards its end. Numerical parameters of 14 analysed calls of two individuals are as follows: call duration (= note du-

ration) 83–118 ms (102.8 ± 10.8 ms); inter-call interval 21–62 ms (42.8 ± 10.7 ms); dominant frequency 3372–3562 Hz (3472 ± 86 Hz); prevalent bandwidth 3200–3800 Hz. Two call series had a duration of 2333 and 3422 ms, and contained 17 and 25 calls, respectively. Call rate within series ranged approximately from 390–420 calls/minute (Fig. 8).

Discussion
Diversification within the subgenus *Gephyromantis*

In this study we complemented previous DNA barcoding data (Vieites et al. 2009, Perl et al. 2014) for the subgenus *Gephyromantis* with a substantially extended set of mitochondrial and nuclear DNA sequences, confirming the presence of numerous species-level evolutionary lineage in this clade of frogs. Besides validating and scientifically naming previously identified candidate species, our more comprehensive field sampling and genetic screening also discovered several additional new lineages such as *G. mafifeo,* and the subsets I and V (currently included in *G. mitsinjo* and *G. sergei*).

There are still several unresolved questions regarding the taxonomy of these frogs that future studies will need to address. These include: (1) The precise genetic identity of *G. leucocephalus*, which can be determined through DNA sequencing of topotypical material and lectotype designation. Further analyses of bioacoustics and morphology are needed to determine whether the current understanding of *G. leucocephalus* as a single species is accurate or if it may consist of multiple species. (2) The status of a candidate species in the *G. decaryi* complex (subset IX, not discussed herein) needs to be clarified. This population exhibits low genetic divergence to *G. hintelmannae* but shows some bioacoustic differences such as shorter advertisement calls (Wollenberg et al. 2012); more sampling in between the respective localities, Manakara and Ambohitsara, is needed. To understand whether (3) the subsets I and II of *G. mitsinjo*, and (4) the subsets IV and V of *G. sergei* represent independent evolutionary lineages respectively, and thus distinct species, analyses of genetic admixture in zones of sympatry with population genomic approaches will be highly informative. The situation is particularly convoluted where subsets IV and V do not appear to form a clade in the mitochondrial gene tree, yet are here both included in one species (*G. sergei*) while subset VI is considered as separate species (*G. mafifeo*) despite being phylogenetically nested within *G. sergei*. We chose this taxonomic solution because no consistent differences were observed between subsets IV and V in terms of bioacoustics, morphology, or nuclear genes. Additionally, the support for the non-monophyly of IV+V was relatively weak, and subset VI differed in terms of nuclear DNA (no shared haplotypes) and morphology (smaller body size). This classification should be considered preliminary until further research using genomic data sets can investigate gene flow, hybridisation, and introgression among these lineages. (5) The

delimitation within the *G. boulengeri* / *G. kremenae* cluster should be refined. Our decision to consider these two entities as distinct species relies mainly on their strongly divergent advertisement calls; however, we also observed non-negligible genetic variation within both species, a certain bioacoustic differentiation within *G. boulengeri* (i.e., calls from Betampona had a less distinct pulsatile structure and were repeated at a faster rate than those from Mahasoa), and a poorly supported mitochondrial monophyly of *G. boulengeri.* Future phylogenomic work will help clarify the exact species boundaries of these two species. (6) Finally, there are several sampling gaps in the molecular data, e.g., in the highlands of the South East and Southern Central East where additional lineages of the *G. blanci* complex may occur, and in the coastal areas of the Southern Central East and South East which may yield further new species of the *G. decaryi* and *G. boulengeri* complexes.

Despite intensive fieldwork in northern Madagascar, no representative of the subgenus *Gephyromantis* has yet been recorded from this part of the island. This is biogeographically relevant because several other subgenera have their center of diversity, and probable center of diversification in northern Madagascar, in particular *Duboimantis* (e.g., Kaffenberger et al. 2012). In contrast, the most basal splits in the phylogeny of the subgenus *Gephyromantis* successively separate clades of species predominantly occurring in the South East and Southern Central East, as suggested by our 16S tree (Fig. 2) and by multigene phylogenetic analyses (Kaffenberger et al. 2012). It is therefore probable that these southern regions correspond to the origin and center of diversification of this group of anurans. An inverse pattern (possible southwards expansions from a northern origin) has been previously observed in two reptile species: the ground chameleon *Brookesia superciliaris* and the gecko *Uroplatus phantasticus* (Ratsoavina et al. 2010, 2012). Also in these two species, a high degree of microendemism was observed, with deep haplotype lineages occupying limited ranges in the eastern rainforests.

Our data also agree with numerous other recent studies (e.g., Köhler et al. 2015, Brown et al. 2016, Ratsoavina et al. 2017, Rakotoarison et al. 2019, Raselimanana et al. 2020) suggesting elevational specialisation in Madagascar's rainforest herpetofauna. This elevational specialisation in *Gephyromantis* apparently characterises major clades, with the four species complexes distinguished herein mostly homogeneous in distribution: *G. decaryi* complex, mostly lowlands < 700 m (except for *G. decaryi* occurring in mid-elevations); *G. blanci* complex, mostly highlands > 1000 m (except *G. enki* whose range extends into lowlands at Ambohitsara); *G. eiselti* complex, mid-elevation sites; *G. boulengeri* complex, mostly lowlands < 700 m, except *G. mitsinjo* that occurs in mid-elevations. Closely related species mostly appear to occupy similar elevational ranges, although some specialisations may occur such as at Pic d'Ivohibe where *G. blanci* was found at a higher elevation than *G. cornucopia*, or in Ranomafana where *G. runewsweeki* occurs at higher elevations than *G. feomborona*.

The accumulating knowledge on phylogeny, genetic differentiation, and advertisement calls of *Gephyromantis* species characterises this group as a fascinating model for the understanding of bioacoustic divergences. This subgenus contains species of low to moderate 16SrDNA distances of 2.5–2.7% and with drastically divergent calls, occurring in close syntopy (*G. eiselti* and *G. thelenae*; Glaw & Vences 2002, Wollenberg & Harvey 2010), allopatric species of rather high genetic distances but barely distinguishable bioacoustically (e.g., *G. sergei* and *G. mitsinjo*: 6.6–7.1%; or *G. sergei* and *G. kremenae*, 6.7–7.3%), as well as species phylogenetically and geographically nested within a clade of bioacoustically static lineages, but highly divergent in call variables (*G. boulengeri* vs. *G. mitsinjo* and *G. kremenae*). Behavioural experiments, e.g., phonotaxis of females vs. conspecific and heterospecific calls, are exceedingly rare in Malagasy anurans (Wollenberg & Harvey 2010, Lam et al. 2020) but constitute a promising future field of study to understand the processes underlying the divergences of *Gephyromantis* frogs, especially if coupled with denser sampling at the contact zones between the different species, and with population genomic analyses of gene flow.

Vulnerability assessment

A large proportion of Madagascar's amphibian species are threatened with extinction (Andreone et al. 2005, 2008) and the genus *Gephyromantis* is no exception. According to the Red List of the International Union International Union for Conservation of Nature (IUCN 2016), in the *G. blanci* and *G. boulengeri* complexes nominal species are currently listed as follows: *G. blanci*, Near Threatened (NT), *G. boulengeri*, Least Concern (LC), *G. enki* and *G. runewsweeki*, both Vulnerable (VU). Our taxonomic revision yielded six new species whose status has so far not been assessed. Furthermore, the new information substantially modified range information for *G. blanci* and *G. boulengeri*, thus requiring a re-assessment of these two species. Unlike several other Malagasy rainforest frogs, many species in the subgenus *Gephyromantis* are found at forest edges or in somewhat degraded forests, and are thus not expected to be immediately threatened by low-level habitat disturbance (e.g., Wollenberg et al. 2012).

For *G. blanci* the distribution area is now limited to two protected areas (Andringitra National Park and Pic d'Ivohibe Special Reserve). Continuous habitat loss likely occurs throughout its range, but without in-depth assessment a change of its current NT category does not seem to be warranted. *Gephyromantis cornucopia* is known from a relatively wide range that includes at least two protected areas (Andohahela National Park and Pic d'Ivohibe Special Reserve). Large intact blocks of mid-elevation forest still exist in Andohahela and the Anosy Mountain Chain, but anthropogenic pressure on these habitats will probably increase in the near future, which would make the species potentially fall into the VU category under criteria B1a and B1biii (range less than 20,000 km², less than 10 known lo-

cations, and continuing decline in the area, extent and/or quality of habitat; IUCN Standards and Petitions Subcommittee 2022); therefore we suggest to classify the species for the time being as NT, similar to *G. blanci*. The third species in the *G. blanci* complex, *G. feomborana*, is only known from two nearby sites in Ranomafana National Park, similar to the situation of *G. runewsweeki;* therefore we propose to classify it as VU, based on criterion D2 (population with < 5 locations, being prone to the effects of human activities or stochastic events within a very short time period in an uncertain future).

In the *G. boulengeri* complex, our data suggest a much smaller range of *G. boulengeri* than in previous taxonomic schemes. At present, the species is only known from one protected area (Betampona Strict Nature Reserve; Rosa et al. 2012). Lowland forest within the range of this species is under high pressure of ongoing deforestation. So far, the species has not been recorded in secondary habitat, and furthermore the population might be threatened by the introduced toad, *Duttaphrynus melanostictus*, that is spreading within its range (Licata et al. 2020). Therefore it seems to be appropriate to classify this species as EN or CR according to criteria B1a and B1biii. The newly described species, *G. mitsinjo, G. kremenae*, and *G. sergei* are each found in at least one major protected area (e.g., *G. mitsinjo*: Mantadia-Analamazaotra National Park; *G. kremenae*, Masoala National Park, Nosy Mangabe Special Reserve; *G. sergei*: Ranomafana National Park) and are not uncommon there. For *G. mitsinjo* and *G. sergei*, our records suggest a certain tolerance of habitat degradation. While a reassessment of these species (along with the required in-depth taxonomic study) might lead to their categorisation as NT or VU, we, for now, suggest keeping a LC status for all three, in line with the status previously assigned to *G. boulengeri* sensu lato and with the commonness of these species across their range, and occurrence in protected areas – although also a NT status could be warranted due to their relatively small ranges and ongoing reduction of suitable habitat. Finally, *G. mafifeo* is currently only known from its type locality which is in no protected area and did not consist of pristine forest. Thus, it can be assumed that the species has a certain tolerance to habitat degradation, and a range likely wider than currently known. Yet, given the extremely limited available information, we propose classifying it as Data Deficient (DD). Considering our preliminary assessments, 41% (7/17) of the species in the subgenus *Gephyromantis* are in a threatened Red List category, while with the previous classification, the percentage was 55% (6/11, IUCN 2015). This exemplifies how taxonomic revision and splitting of widespread species into smaller-range species-level units does not necessarily lead to exacerbated threat levels (see for instance Scherz et al. 2019), as in the case in our study, three of the new species were split from a previous LC species (*G.kremenae, G. mitsinjo, G. sergei* from *G. boulengeri*) and, in a preliminary way, a continued LC (or NT) classification may apply to all of them. Nevertheless, given the ongoing dramatic habitat destruction affecting most of Madagascar's remaining forests, it is likely that

the proportion of threatened species in the subgenus *Gephyromantis* will soon increase. In-depth taxonomic study continues to be of paramount importance to identify priority areas for conservation which may exist even in areas usually not taken into account, such as in the presumed range of *G. mafifeo*.

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Supplementary data

The following data are available online:

Supplementary document 1. GenBank accession numbers of sequences used in this study.

Supplementary document 2. Morphological dataset.

Supplementary document 3. Bivariate plot of the SVL and TL (females excluded).

Supplementary document 4. Exploratory PCA of the morphological dataset.