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Taxonomy and geographic distribution of Malagasy frogs of the *Gephyromantis asper* clade, with description of a new subgenus and revalidation of *Gephyromantis ceratophrys*

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Abstract. We integrate molecular, bioacoustic and morphological data to provide a systematic revision of the frogs classified in the *Gephyromantis asper* clade (Anura: Mantellidae), endemic to Madagascar. Based on concordant differentiation in a mitochondrial and a nuclear gene (16S rRNA and Rag1) we distinguish six different species in this clade: *G. ambohitra, G. asper, G. tahotra, G. spinifer, G. ceratophrys* bona species, and an undescribed species. *Gephyromantis ceratophrys* (Ahl, 1929) is resurrected from the synonymy of *G. asper* and refers to the southernmost populations previously assigned to that species (i.e., from Ranomafana National Park in the southern central east of Madagascar). We provide several new geographical records verified by molecular sequence identity: *G. asper* is confirmed from the localities Anjozorobe, Mandraka, and Tsinjoarivo; *G. spinifer* is confirmed from Pic Ivohibe, a record previously considered questionable and representing the northernmost locality known for this species; *G. ambohitra* is recorded from the Masoala Peninsula, extending its known distribution range eastward; and *G. tahotra* (previously only known from its type locality Marojejy) is confirmed for the Tsaratanana and Sorata Massifs. An undescribed species of the group occurs at sites south and southwest of the Tsaratanana massif. It is differentiated in mitochondrial and nuclear DNA, but no bioacoustic and insufficient morphological data are thus far available for its formal taxonomic description. Because species in the *G. asper* clade, within *Gephyromantis*, are phylogenetically distant from other species of the subgenus *Duboimantis*, we propose a new subgenus *Asperomantis* for this clade of Malagasy rainforest frogs. Most individuals belonging to this subgenus are easily distinguished from other species in the genus *Gephyromantis* and most other mantellids by the presence of a light spot in or near the centre of the tympanum.

Key words. Amphibia, Anura, Mantellidae, *Gephyromantis*, systematics, bioacoustics, 16S rRNA, Rag1, *Gephyromantis ceratophrys* bona species, *Asperomantis*, new subgenus.

Introduction

The genus *Gephyromantis* Methuen, 1920 forms part of the endemic Malagasy-Comoroan anuran family Mantellidae and currently contains 41 species in five subgenera (Glaw & Vences 2006, Wollenberg et al. 2011, Kaffenberger et al. 2012). *Gephyromantis* are terrestrial or scansorial frogs typically found in rainforests, but also in dry

forests (Crottini et al. 2011), and often exhibit a derived larval morphology. Some species are characterized by carnivorous tadpoles (Reeve et al. 2011), whereas others have non-feeding endotrophic tadpoles, developing either in small streams or terrestrial nests (Randrianiaina et al. 2011). Several subgroups of *Gephyromantis* have traditionally been thought to be characterized by direct development, in particular the frogs related to *G. asper* (previously *Mantidactylus asper*) for which Blommers-Schlösser (1979) reported direct development on the basis of eggs found next to a calling male and therefore assigned to this species. However, subsequent descriptions of tadpoles identified by DNA barcoding (Randrianiaina et al. 2007, 2011) provided evidence that generalized, exotrophic tadpoles characterized *G. asper* and various related species, suggesting that these species do not in fact have endotrophic larvae nor a direct development.

Gephyromantis asper was assigned to the subgenus *Duboimantis* Glaw & Vences, 2006, which generally comprises small to medium-sized rainforest frogs that are found on the forest leaf litter during the day, and call from perched positions at night. Within *Duboimantis*, *G. asper* forms a clade together with several described species (*G. spinifer, G. ambohitra, G. tahotra*) and potential candidate species (*G.* sp. aff. *asper, G.* sp. Ca28 and *G.* sp. Ca27; Vieites et al. 2009, Kaffenberger et al. 2012, Perl et al. 2014). We here refer to this clade as the *Gephyromantis asper* clade.

The most recent phylogenetic assessments, based on a combination of mitochondrial and nuclear genes, have failed to provide support for the monophyly of *Duboimantis* (Vieites et al. 2009, Wollenberg et al. 2011, Kaffenberger et al. 2012, Pyron & Wiens 2011, Perl et al. 2014). According to the most species- and data-rich analysis to date (Kaffenberger et al. 2012), the species included in this subgenus instead form two highly supported clades of uncertain positions within the genus *Gephyromantis*: the *Gephyromantis asper* clade as defined above, and a clade with all other *Duboimantis* species including the type species of the subgenus (*G. granulatus*; Glaw & Vences 2006).

Since the last revision of the *G. asper* clade (Vences & Glaw 2001), taxonomic progress has been impeded by the lack of substantial new field data. Tissue samples of *G. spinifer* were scarce and an assessment of this species' molecular variation thus impossible. Bioacoustic data from genetically divergent populations assigned to *G. asper* from the southern central east of Madagascar were not available either, rendering an integrative assessment of the status of these morphologically cryptic populations very difficult. One new species, *G. tahotra*, was described more recently from the Marojejy massif in northeastern Madagascar (Glaw et al. 2011), but its distribution could not be ascertained due to overall sparse sampling from northern Madagascar.

Here we provide an updated and revised taxonomy of the *Gephyromantis asper* clade on the basis of newly collected specimens, new bioacoustic recordings, and new DNA sequencing. We summarize molecular data from a mitochondrial and a nuclear gene, analyses of advertisement calls, and morphological comparisons to characterize the species of this clade and their geographic ranges. We redefine *G. asper,* resurrect a synonym (as *G. ceratophrys*), and propose a new subgenus for the species of the *G. asper* group based on previous phylogenetic findings.

Materials and methods

Individuals were collected mostly at night by searching for calling males, using headlamps, and opportunistically during forest walks at day and night. They were euthanised by an overdose of MS222 or chlorobutanol solution, subsequently fixed in 95% ethanol or 5% formalin, and preserved in 70% ethanol. Tissue samples (typically, muscle tissue taken ventrally from one thigh) were taken in the field and separately preserved in 99% ethanol. Locality information was recorded with GPS receivers. Vouchers were deposited in the collections of the Mention Zoologie et Biodiversité Animale, Faculté des Sciences, Université d'Antananarivo, Antananarivo (UADBA), Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK), Zoologische Staatssammlung München (ZSM), and Museo Regionale di Scienze Naturali di Torino (MRSN). FGMV, FGZC, and ZCMV refer to F. Glaw and M. Vences field numbers, respectively. ACP refers to a sample number of A. Crottini. Additional material from The Natural History Museum, London (BM = BMNH) and Museum für Naturkunde, Berlin (ZMB) was examined. In addition we also report on specimens from MNHN (Muséum National d'Histoire Naturelle, Paris), MTKD (Museum für Tierkunde, Dresden), and ZMA (Zoological Museum Amsterdam).

We analysed one segment of mitochondrial DNA (mtDNA) corresponding to the 16S rRNA gene (16S), and one segment of nuclear DNA (nucDNA) corresponding to the recombination-activating gene 1 (Rag1). DNA was salt-extracted from tissue samples following standard protocols as described in Kaffenberger et al. (2012). Sequences were retrieved from GenBank or newly generated.

We amplified a segment of the 16S rRNA gene widely used for barcoding and taxonomy in Malagasy amphibians (Vences et al. 2005, Vieites et al. 2009), using two primer pairs: AC16SAR-L/AC16SBR-H (Crottini et al. 2014) and 16SFrogL1/16SFrogH1 (Vences et al. 2010). We then amplified a segment of Rag1 using a nested approach with PCR conditions as detailed in Rakotoarison et al. (2015), using first the primer pair Rag1-Mart-F1 and Rag1-Mart-R6, and subsequently Rag1-Amp-F2 and Rag1-UC-R (for details of primer sequences, original sources, and PCR protocols, see Rakotoarison et al. (2015)). Purification of PCR products was carried out with Exonuclease I and Shrimp Alkaline Phosphatase digestion. Amplicons were sequenced with the PCR primers, using BigDye v. 3.1 cycle sequencing chemistry. Sequencing products were run on a 3130xl genetic analyzer (Applied Biosystems) and assembled and quality-checked in CodonCode Aligner v. 3.0.3. All DNA sequences newly generated in this study were deposited in

GenBank (accession numbers KY412924-KY412964 for 16S rRNA and KY406180-KY406227 for Rag1).

Sequences of both gene segments were aligned using the ClustalW algorithm implemented in MEGA v. 6.0 (Tamura et al. 2013). Two outgroup sequences from *G. pseudoasper* and *G. redimitus* were included in the 16S rRNA alignment; twelve indels were removed. MEGA was used to calculate genetic distances between and within groups. For analyses of the 16S rRNA gene fragment, the optimal model of nucleotide substitution was selected using the Akaike information criterion corrected for small sample size (AICc) after computing likelihood scores for 24 models in jModelTest v. 2.1.4 (Darriba et al. 2012). We conducted a Bayesian Inference phylogenetic analysis (BI) in MrBayes v. 3.2 (Ronquist et al. 2012) with parameters of the likelihood and phylogenetic settings set to the optimal nucleotide substitution model (SYM+G). The Markov chain Monte Carlo included two runs with four chains sampled every 10^3 generation for a total of 10^6 generations; the first 25% of the sampled generations were discarded as burn-in. We checked convergence between runs by examining the average standard deviation of split frequencies and parameter sampling in Tracer v. 1.5 (Rambaut et al. 2013) and the 'compare' function of AWTY (Nylander et al. 2008). We also ran a heuristic maximum likelihood (ML) analysis in MEGA with 1000 bootstrap replicates. As SYM cannot be specified in MEGA, we used the HKY + G model of sequence evolution that had the highest AICc support of all implementable models.

We used a comparatively short segment of the Rag1 gene (505 bp) to maximize the number of samples included, given that for some samples, no high-quality reads could be obtained for the entire gene segment originally amplified, and sequences must not include missing data for network analyses. After the detection of heterozygous nucleotide sites in the Rag1 dataset, we applied the software Phase v. 2.1.1 (Stephens et al. 2001, Stephens & Scheet 2005) to infer haplotypes. The relationships of the Rag1 haplotypes within and between species of the *G. asper* group were then examined in a statistical parsimony framework with a 95% cut-off with the aid of the software TCS v. 1.21 (Clement et al. 2000).

We also examined the phylogenetic affinities of *G.* sp. Ca28, a lineage first detected by Perl et al. (2014), but not analysed by Kaffenberger et al. (2012), and here provide genetic and morphological data for it. We inserted the sequences of three genes of this candidate species (16S and Rag1 acquired in this study; COI downloaded from GenBank, KF611486; all other genes coded as missing data) into the multigene analysis of Kaffenberger et al. (2012). A BI was conducted using the same partitioning strategy, models of nucleotide substitution, priors, and parameter values as in Kaffenberger et al. (2012). Convergence between runs was checked in Tracer and AWTY.

Morphometric measurements were either obtained from previous studies (Vences & Glaw 2001, Glaw et al. 2011) or newly taken to the nearest 0.1 mm with a precision calliper from more recently collected specimens by MV. The following characters were measured: snout–vent length (SVL), maximum head width (HW), head length from posterior maxillary commissure to snout tip (HL), horizontal eye diameter (ED), horizontal tympanum diameter (TD), distance from eye to nostril (END), distance from nostril to snout tip (NSD), distance between nostrils (NND), foot length (FOL), foot length including tarsus (FOTL), hindlimb length from cloaca to tip of longest toe (HIL), forelimb length from axilla to tip of longest finger (FORL), and length and width of femoral gland (FGL, FGW). To allow direct comparisons, the webbing formulae follow the system that was first applied to Madagascan frogs by Blommers-Schlösser (1979) and adopted by most subsequent authors. Femoral gland terminology and examination follows Glaw et al. (2000); 'internal view of femoral glands' refers to examination after dissection and reflection of the ventral skin of a shank in preserved specimens.

All statistical analyses were carried out with Statistica, v. 7.1 (StatSoft, Tulsa, USA), using measurements that were size-adjusted as ratios to SVL. We performed a multivariate analysis of variance with species as categorical predictors and tested for differences between pairs of species using Tukey's post-hoc tests separately for males and females.

Vocalizations were recorded in the field using different types of recording devices (Sony WM-D6C, Tensai RCR-3222, Edirol R-09) and built-in or external microphones (Sennheiser Me-80, Vivanco EM 238). Recordings were sampled or re-sampled at 22.05 kHz and 32-bit resolution and computer-analysed using the software Adobe Audition v. 1.5. Frequency information was obtained through Fast Fourier Transformations (FFT; width 1,024 points). Spectrograms were obtained with the Hanning window function at 256-bands resolution. Temporal measurements are given as mean ± standard deviation with range in parentheses. Terminology in call descriptions generally follows Köhler (2000).

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: urn:lsid:zoobank.org:pub:D6667E29-361F-429D-A906-2FA79F31E454. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: www.salamandra-journal.com.

Results

Molecular differentiation

We obtained DNA sequences from altogether 76 individuals for the target taxa not including the outgroups for the 16S rRNA alignment (458 bp excluding indels), including 41 new sequences and 35 downloaded from GenBank. A total of 96 nucleotides were variable, 92 of which were parsimony informative (excluding outgroups). Phylogenetic analysis of mtDNA sequences of the 16S rRNA gene result-

ed in Bayesian and maximum likelihood topologies that were very similar except for minor rearrangements within major clades (Fig. 1). Although many of the nodes of this tree were unsupported due to the small size of the mtDNA segment, we could still confidently discern seven main lineages (Fig. 1), separated from each other by long branches and characterized by low intra-clade differentiation. These lineages corresponded to (i) *Gephyromantis ambohitra* from its type locality Montagne d'Ambre, (ii) *G. ambohitra* from Manongarivo and the Masoala peninsula (Amparihy), (iii) *G. tahotra* from its type locality Marojejy plus several other, new localities, (iv) *G. spinifer* from southeastern Madagascar, (v) sequences from Ranomafana, corresponding to a population referred to as to *G.* sp. aff. *asper* by Kaffenberger et al. (2012) and *G. asper* by Vieites et al. (2009) and Perl et al. (2014), (vi) populations from Mandraka and several other localities, assigned to *G. asper* by Blommers-Schlösser (1979) and Kaffenberger et al. (2012), but to *G.* sp. Ca27 by Vieites et al. (2009) and Perl et al. (2014), and (vii) three specimens from two sites in northern Madagascar, corresponding to *G.* sp. Ca28 as first defined by Perl et al. (2014) (see Fig. 2 for the geographic distribution of these lineages). Sequence divergences (uncorrected pairwise p-distances; Table 1) between these main lineages were between 4.2 (*G.* sp. Ca27 vs Ca28) and 12.2% (*G. ambohitra* vs *G. tahotra*). For improved comparison and consistency between text and figures and tables, we here anticipate our taxonomic conclusions and in the following will refer to the Mandraka lineage (vi) as *G. asper*, and to the Ranomafana lineage (v) as *G. ceratophrys*, thus implying a change in taxonomic assignment compared to Vieites et al. (2009), Kaffenberger et al. (2012), and Perl et al. (2014).

We also newly obtained 505 bp for 48 samples from the 5' end of the Rag1 gene. This segment was highly variable with 40 polymorphic sites, 18 of which were heterozygous in at least one individual. Phasing resulted in high probability (1.00) haplotype pairs for all but 5 sequences. The

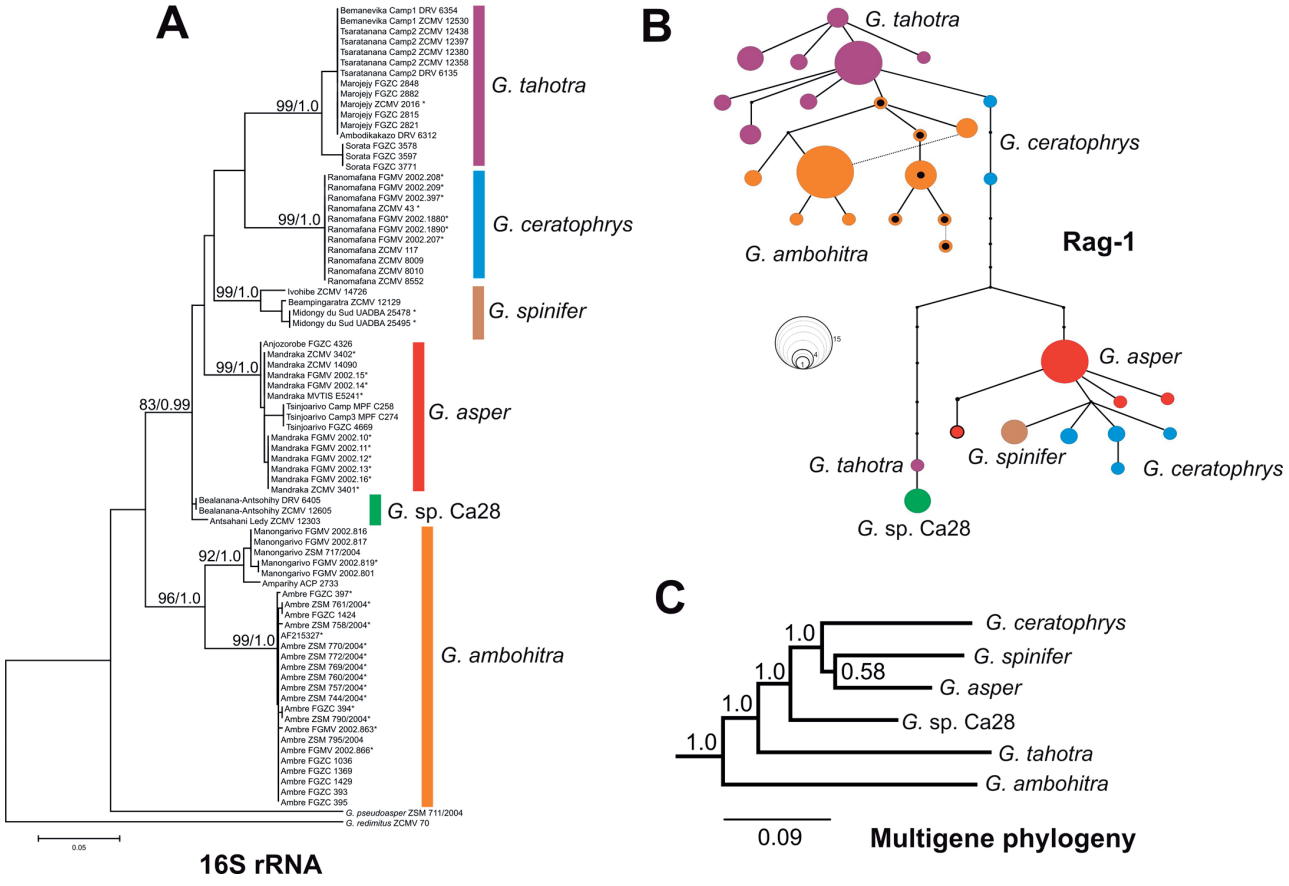

Figure 1. (A) Maximum likelihood tree of species in the subgenus *Asperomantis* based on a 458-bp segment of the mitochondrial 16S rRNA gene. The numbers at the nodes indicate ML bootstrap support and Bayesian posterior probabilities obtained from a Bayesian phylogenetic analysis. Only values for major nodes with at least 80% bootstrap support and 0.8 posterior probability are shown. Asterisks denote sequences downloaded from Genbank. (B) Maximum parsimony network of the subgenus *Asperomantis* based on a 505-bp segment of the Rag1 gene. The circle diameter is proportional to the sample size, colour coding as in (A). The dotted symbols within circles in *G. ambohitra* represent haplotypes found only in the Manongarivo population. (C) Phylogenetic relationships within the subgenus *Asperomantis* based on a multigene Bayesian analysis (posterior probabilities above nodes). This is an excerpt from a tree for all *Gephyromantis* frogs based on methods described in Kaffenberger et al. (2012). *Gephyromantis ceratophrys* and *G. asper*, as depicted in this figure, correspond to *G. asper* and *G.* sp. Ca27, respectively, of Vieites et al. (2009) and Perl et al. (2014).

divergences ranged from 0.7 (3.6 substitutions on average) between *G. asper* and *G. spinifer* to 2.7% (13.8 substitutions on average) between *G. ambohitra* and *G.* sp. Ca28. The divergence in nucDNA was in many aspects concordant with the signal observed in mtDNA, i.e., we found no instances of haplotype-sharing in this segment of the Rag1 gene between any of the species (Fig. 1B). Interestingly, we did not detect haplotype-sharing between *G. ambohitra*

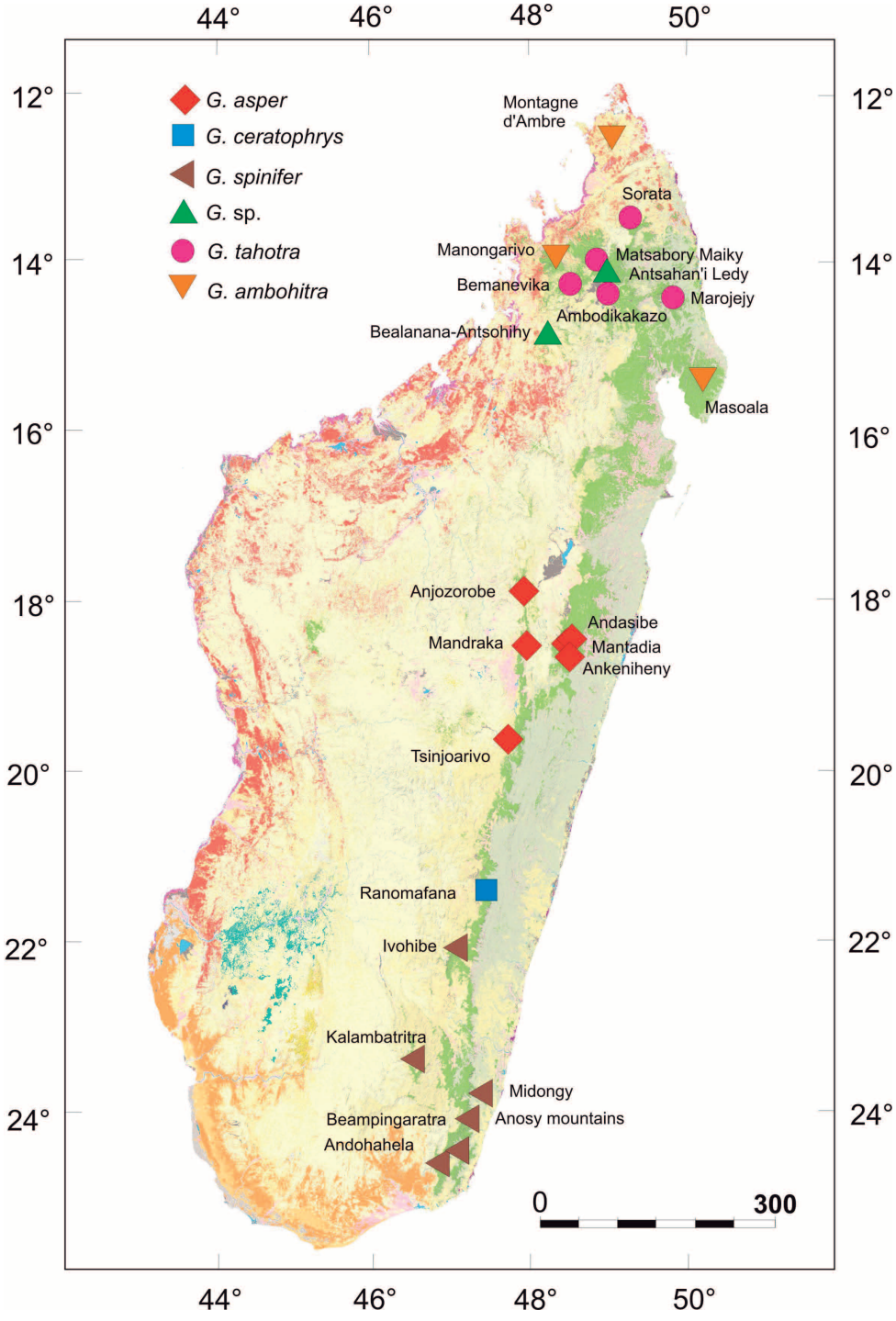

Figure 2. Map showing the confirmed distribution records of all six species in the subgenus *Asperomantis*. The map also shows the remaining primary vegetation of Madagascar (www.vegmad.org), with green colours indicating rainforest, reddish colours deciduous dry forest, and orange colour arid spiny forest. All localities were confirmed by DNA sequences except for the Anosy Mountains (type locality of *G. spinifer*), Kalambatritra (*G. spinifer*), Andasibe, Ankeniheny and Mantadia (localities of *G. asper,* assigned by morphology and/or bioacoustics).

Table 1. Estimates of evolutionary divergence between taxa of the subgenus *Asperomantis*. The number of base-differences per site from averaging over all sequence pairs between groups (p-distance) in the 16S rRNA (458 positions, below diagonal) and Rag1 (505 positions, above diagonal) alignments. Average values within group distances for the 16S rRNA gene are placed on the diagonal (bold italics). All positions containing indel polymorphisms were excluded.

	G. ambohitra	*G.* sp. Ca28	*G. asper*	*G. spinifer*	*G. ceratophrys*	*G. tahotra*
G. ambohitra	***0.022***	0.027	0.017	0.023	0.020	0.009
G. sp. Ca28	0.078	***0.007***	0.021	0.024	0.024	0.024
G. asper	0.104	0.042	***0.005***	0.007	0.009	0.017
G. spinifer	0.094	0.047	0.062	***0.013***	0.008	0.021
G. ceratophrys	0.119	0.062	0.078	0.075	***0.000***	0.018
G. tahotra	0.122	0.068	0.069	0.082	0.074	***0.006***

from Montagne d'Ambre and Manongarivo either. Most haplotypes of each species grouped closely together in the network. However, *G. ceratophrys* contained two distinct groups of haplotypes, while one *G. tahotra* haplotype was closely related to the single haplotype found in *G.* sp. Ca28 (Fig. 1B) and distant from all other *G. tahotra* haplotypes. Sequences of *G. tahotra* formed a separate haplotype group with haplotype-sharing observed between most populations of this species. A third main haplotype group was formed by sequences of *G. asper* (from Mandraka, Anjozorobe and Tsinjoarivo), *G. ceratophrys* (the Ranomafana mtDNA lineage), and *G. spinifer*.

Our implementation of the multigene Bayesian analysis of Kaffenberger et al. (2012) with the inclusion of *G.* sp. Ca28 resulted in the placement of this taxon as the sister group to a clade composed of *G. ceratophrys*, *G. spinifer* and *G. asper*. All other phylogenetic relationships were consistent with those reported by Kaffenberger et al. (2012).

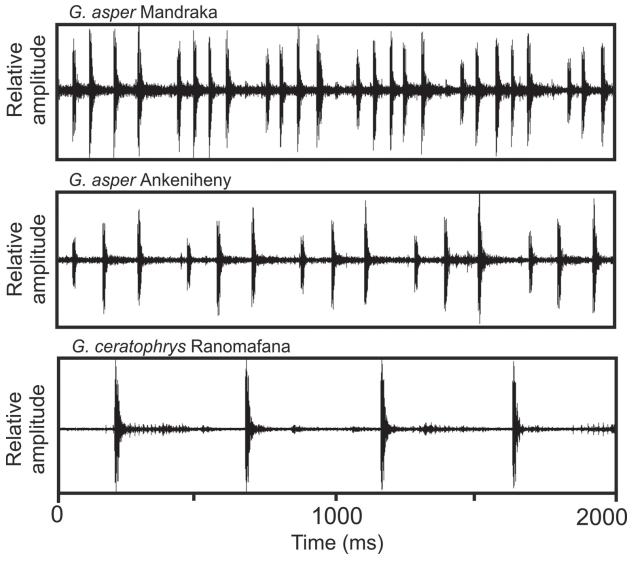

Figure 3. Comparative oscillograms of sections of the calls of *Gephyromantis asper* and *G. ceratophrys* (2,000 ms sections each). Each call section refers to an individual frog. Notes in *G. asper* are arranged in note groups, with increasing intensity of notes in one group, while *G. ceratophrys,* even in dense choruses, was only heard emitting single notes in regular series.

Bioacoustic differentiation

Call recordings from previous studies (Vences & Glaw 2001, Glaw et al. 2011) and new fieldwork (partly included in Vences et al. 2006) were available for *G. ambohitra* (both from Montagne d'Ambre and Manongarivo), *G. tahotra* and for *G. asper* and *G. ceratophrys*; no call data exist for

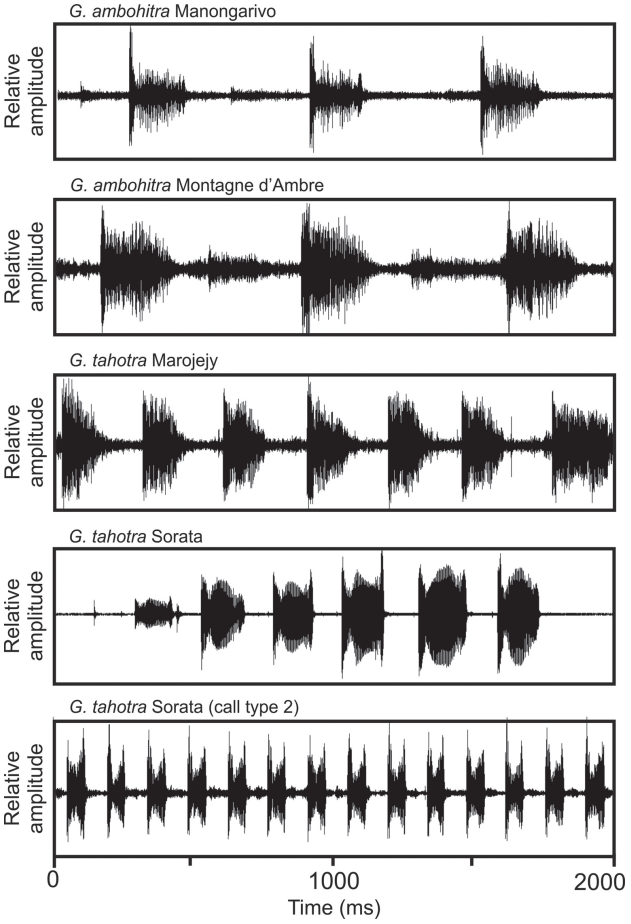

Figure 4. Comparative oscillograms of sections of the calls of *Gephyromantis ambohitra* and *G. tahotra* (2,000 ms sections each). Each call section refers to an individual frog. See call description of *G. tahotra* and Table 2 for further explanations and comparison.

Table 2. Comparative advertisement call parameters of species of the subgenus *Asperomantis*. *) Recording obtained by R. Blommers-Schlösser. Sample size refers to the number of calls (not number of individual frogs).

Species Locality Sample size	Note duration [ms]	Pulses/second within notes	Inter-note interval [ms]	Dominant frequency range [Hz]	Dominant frequency peak [Hz]	Recording temperature [°C]
G. asper Ankeniheny (n = 10)	10±3 (5–13)	–	66±11 (56–90)	1700–7700	3269±207 (3086–3614)	23.5
G. asper Mandraka (n = 15)	20±5 (12–26)	–	69±26 (41–122)	1600–6500	3426±284 (3014–3919)	18.4
G. asper Mandraka* (n = 7)	16±2 (13–20)	–	75±2 (72–80)	1800–6000	–	–
G. ceratophrys Ranomafana (n = 15)	28±6 (20–44)	–	561±202 (428–912)	1600–8000	3844±31 (3811–3878)	estimated 20–22
G. ambohitra Montagne d'Ambre (n = 12)	230±35 (152–274)	ca 150	475±24 (447–509)	2800–4400	3255±48 (3180–3366)	21.0
G. ambohitra Manongarivo (n = 17)	206±20 (184–254)	ca 160	481±83 (376–643)	1600–4500	3091±28 (3044–3115)	23.0
G. tahotra Marojejy (n = 11)	186±31 (152–266)	ca 310	116±25 (77–157)	1400–5800	1546±10 (1435–1562)	21.0
G. tahotra Sorata (n = 8)	109±9 (98–120)	ca 360	156±12 (143–172)	1400–6000	1457±6 (1449–1468)	–

G. spinifer and *G.* sp. Ca28. Detailed call descriptions are given in the species accounts below, and a summary of call structures is provided in Figs 3 and 4. No major call differences were observed between populations belonging to the same mtDNA lineages. Furthermore, populations of *G. ambohitra* from Montagne d'Ambre and Manongarivo have virtually identical calls, as has previously been reported (Glaw et al. 2011).

The calls of *G. ambohitra* and *G. tahotra* consisted of relatively long notes of 98–274 ms in duration (Fig. 4, Table 2). These were arranged in stereotyped series, with a distinctly faster repetition rate in *G. tahotra.* A second call type with shorter notes (61–71 ms), probably emitted in a territorial context, was also observed in *G. tahotra.* In contrast, long-note calls were never heard in *G. asper* or *G. ceratophrys*. The calls recorded in the respective populations (Fig. 3, Table 2) consist of short notes of < 50 ms in duration. These are emitted as a stereotyped series in *G. ceratophrys,* but arranged into note groups of a very characteristic "galloping pattern" in *G. asper*.

Morphological differentiation

Examination of morphological characters yielded very few differences between the species of the *G. asper* clade, apart from those mentioned previously (Vences & Glaw 2001, Glaw et al. 2011). Original morphometric measurements are given in Appendices 1–2. Values of all measurements overlapped between all species/lineages. The results of our multivariate analyses are presented in Table 3. Significant differences in body size (SVL) were observed between most species both in males and females, except for the comparison between *G. asper* and *G. ceratophrys*. Overall, these two species were characterized by comparatively small body sizes (max. SVL in males and females 24–25 mm vs > 30 mm in *G. ambohitra, G. tahotra*, and *G. spinifer*), and small size might also characterize *G.* sp. Ca28. However, too few specimens were available for a thorough assessment of variation, and it is uncertain whether the specimens available of *G.* sp. Ca28 are fully-grown adults suitable for comparisons of this kind.

A series of other morphometric variables showed significant differences between species (Table 3), but in no case were the respective values diagnostic, i.e., non-overlapping. As a general trend, *G. ambohitra* differs from several other species by its relatively shorter fore- and hindlimbs and a wider head, but few other differences were observed that could be used to distinguish the various species morphologically. In addition to its morphometric characters, *G. spinifer* differs from the other species by having more strongly expressed spines and ridges on the dorsal sides of head and

Table 3. Summary of morphometric differences between species of the subgenus *Asperomantis*. Males below diagonal, females above diagonal. Abbreviations of variables as in Materials and Methods. Variables (except SVL) were size-adjusted as ratios to SVL, and compared by Tukey's post-hoc tests after performing a multivariate analysis of variance. Variables listed are those that showed significant differences ($P < 0.05$) in these pairwise comparisons.

	G. asper	*G. ceratophrys*	*G. spinifer*	*G. ambohitra* M. d'Ambre	*G. ambohitra* Manongarivo	*G. tahotra*
G. asper	–		SVL, NND	SVL, HW, NND, FOTL, FOL	not applicable	SVL
G. ceratophrys	TD	–	SVL	SVL, HW, HL, FORL, HAL, HIL, FOTL, FOL	not applicable	SVL, HL, FORL
G. spinifer	SVL	SVL, TD	–	HW, HAL, HIL, FOTL, FOL	not applicable	TD, END
G. ambohitra M. d'Ambre	SVL, HW, ED	SVL, TD, ED	SVL	–	not applicable	END, HIL, FOTL, FOL
G. ambohitra Manongarivo	SVL, HW, FOTL	SVL, HW, TD	SVL, HW, HIL, FOTL, FOL	SVL, ED, FOL	–	not applicable
G. tahotra	SVL, HW	SVL, TD		SVL, ED	SVL	–

body (Vences & Glaw 2001), while the weakest expression of these structures is found in *G. ambohitra* and *G. tahotra.* Furthermore, *G. spinifer* differs from all others species by a more contrasting black-white pattern on its ventral side.

Our field observations suggested possible differences in femoral gland morphology. Therefore, we compared femoral glands (present only in males in *Gephyromantis*) between species (Supplementary Table 2, Fig. 5). Relative to body size, the largest femoral glands were found in *G. ceratophrys*, although wide variation of both FGL and FGW was observed within species. The femoral gland morphology allows to distinguish most males of the two small-sized species, *G. asper* and *G. ceratophrys*, because the former has typically less clearly developed glands (Fig. 5). In external view of live specimens (Fig. 5), males of *G. asper* appear to have a larger number of gland granules (single glands packed into the femoral macrogland; Vences et al. 2007), but these can be very indistinct, as is illustrated by the specimen from Ankeniheny pictured in Figure 5. However, in internal view of preserved specimens, often only a few granules are visible in *G. asper.* This paradoxical situation might be caused by the smaller size of these granules: in life, they can often be recognized by their coloration differing from the surrounding skin, but once in preservative, the colour will fade and the granules are not clearly visible by size alone, not even in internal view. In conclusion, both in internal and external view, the femoral glands appear to be more distinct in *G. ceratophrys* than in *G. asper*.

Taxonomic conclusions

Despite a lack of clear morphological differentiation between several of the compared lineages, we find evidence for six or seven distinct species in the *G. asper* clade. *Gephyromantis ambohitra* and *G. tahotra* are well differentiated from each other and from the remaining species by their advertisement calls, concordant differentiation in mtDNA and nucDNA, and by some morphometric differences (especially *G. ambohitra*). Few data are available for *G. spinifer*, but this species is distinct in mtDNA, nucDNA and morphology (strongly expressed spines and ridges, contrasting ventral pattern). We assume that also *G.* sp. Ca28 represents a distinct species as it is differentiated in both mtDNA and nucDNA, but the scarcity of specimens, lack of bioacoustic data, and closeness of the Rag1 haplotype with one individual of *G. tahotra* (Fig. 1) lead us to postpone its formal description.

Lastly, the statuses of the two small-sized lineages from central eastern Madagascar (herein assigned to *G. asper* and *G. ceratophrys*) require careful evaluation. They show distinct and consistent differentiation in mtDNA (mean 16S p-distance of 8.2%) and absence of nucDNA haplotype-sharing. One relevant morphological difference (femoral gland morphology) is present, and their advertisement calls are clearly distinct. These lines of evidence point to distinct species statuses for both of these lineages.

Two early names are available to refer to these two species from central eastern Madagascar: *Rana aspera* Boulenger, 1882, and *Mantidactylus ceratophrys* Ahl, 1929. The type specimens of both had been studied by Vences & Glaw (2001) before and were re-examined for the present study (see Supplementary Table 1 for morphometric measurements). The types of *R. aspera* were collected in the region of East Betsileo while those of *M. ceratophrys* were collected in Betsileo. The Betsileo region is located in the southern central east of Madagascar and includes Ranomafana, the locality of one of the small-sized lineages. However, we found the *G. asper* mtDNA lineage present at Mandraka also at Tsinjoarivo, which almost borders the northern edge of the Betsileo region and is not separated from that region by obvious important geographic

barriers. Therefore, it seems likely that both lineages are present in the Betsileo region. The Mangoro River might contribute to separating *G. asper* (north of the catchment) and *G. ceratophrys* (south of the catchment), given that *Gephyromantis* frogs are affected by riverine barriers (e.g., Wollenberg Valero 2015). However, the effect of this river on this pair of highland species is probably minor, considering that in their area of occurrence, the river is probably not wide enough to act as an efficient barrier, as is known for other frogs (e.g., Gehring et al. 2012).

While the types of *R. aspera* comprise both males and females, those of *M. ceratophrys* are all females and do not exhibit femoral glands. These glands are very small and almost indistinguishable in the males of the *R. aspera* type series, even in internal view (Fig. 5). This character state is also found in some specimens from Ankeniheny and Mandraka, but strongly differs from the specimens occurring at Ranomafana. Based on this character, we here assign the name *Gephyromantis asper* (Boulenger, 1882) to the lineage occurring in Mandraka, Ankeniheny, Andasibe,

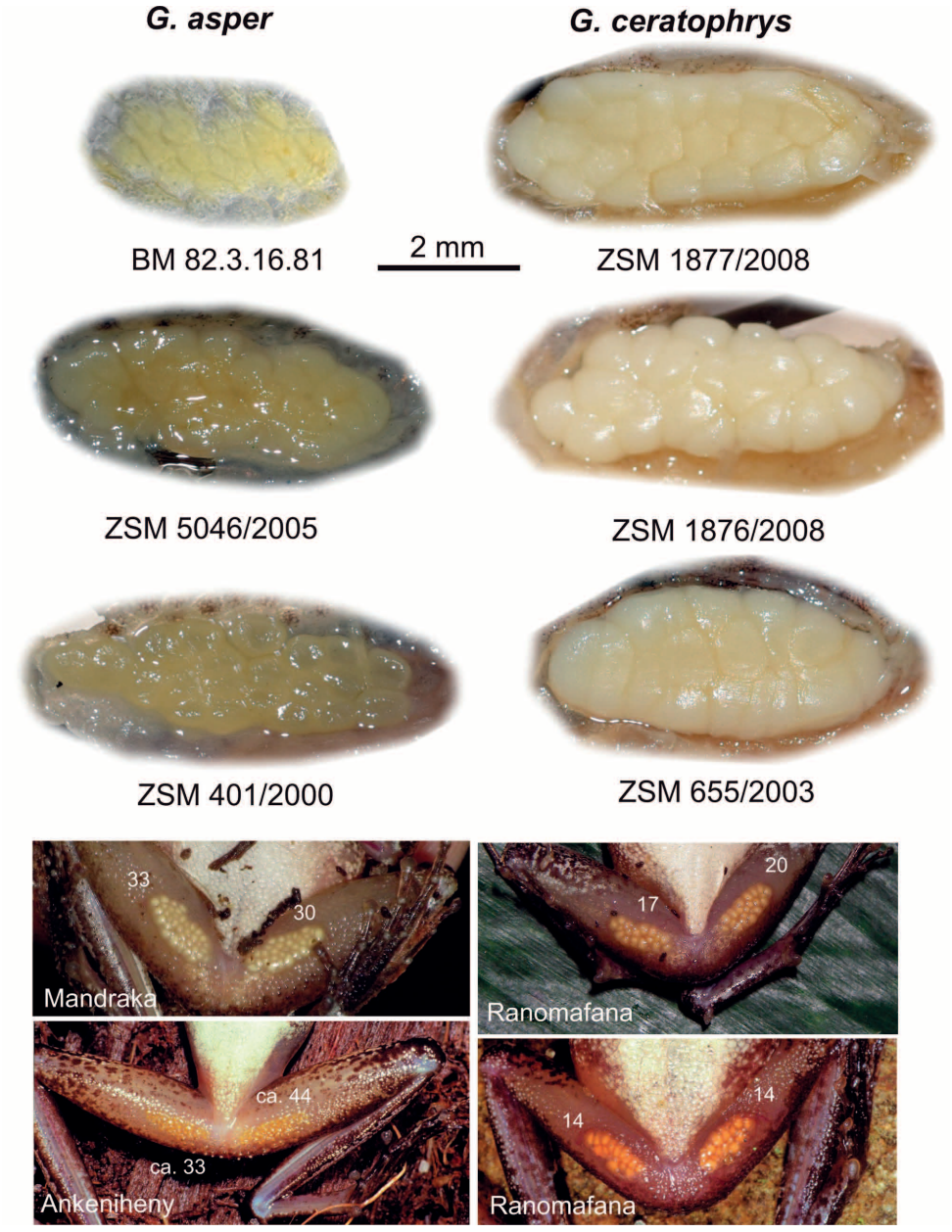

Figure 5. Comparison of femoral gland morphology in males of *Gephyromantis asper* and *G. ceratophrys*. The upper six pictures show glands of preserved specimens in internal view (after reflecting the ventral skin of thigh), the lower four pictures show the thighs in live specimens. Typically, the femoral glands are less strongly expressed and less prominent in *G. asper*, although this is not always true (e.g., the live specimen from Mandraka). In life, the glands of *G. asper* appear to be composed of a larger number of granules (white numbers on the lower four pictures), but only a rather small number of granules is typically recognizable in preserved specimens.

Mantadia, Anjozorobe and Tsinjoarivo, in agreement with Blommers-Schlösser (1979) and Kaffenberger et al. (2012), assuming that the southern distribution border extends or extended into the Betsileo region where the types of *aspera* were collected.

In a second step, we suggest applying the name *Gephyromantis ceratophrys* (Ahl, 1929) to the Ranomafana population, given that (i) there is no morphological difference between the type series and the specimens collected in Ranomafana, and (ii) Ranomafana lies within the Betsileo region, which is the type locality of *M. ceratophrys*. We consider this to be the most plausible and nomenclaturally most parsimonious solution to naming the two identified species of this clade in central eastern Madagascar, without needing to introduce new names.

Subgeneric classification

We base our phylogenetic assessment of relationships within *Gephyromantis* on the study of Kaffenberger et al. (2012) who most comprehensively assessed the phylogeny of this genus, based on a near-complete data matrix of five mitochondrial and five nuclear genes. In their study, the subgenus *Duboimantis* was found to be polyphyletic and consisting of two strongly supported clades in very different positions of the tree. One of these clades corresponded to the *G. asper* clade, which was sister to a clade containing species of the subgenera *Gephyromantis* and *Phylacomantis*. This is supported by other studies based on incomplete data matrices (Pyron & Wiens 2011), single mitochondrial genes (Vieites et al. 2009, Perl et al. 2014), a combination of three mitochondrial genes (Wollenberg et al. 2011), or three mitochondrial and one nuclear marker (Glaw & Vences 2006). The subgenus *Duboimantis* was not recovered as monophyletic in any of these studies, whereas the *G. asper* clade was recovered whenever more than two species of that clade were included.

Randrianiaina et al. (2007, 2011) furthermore provided evidence that the two clades of *Duboimantis* differ in larval development: populations corresponding to the *G. asper* group (known from *G. asper*, *G. ceratophrys*, *G. ambohitra*, and *G. tahotra*) have generalized, exotrophic tadpoles, whereas populations of the second *Duboimantis* clade (known from *G. granulatus*, *G. sculpturatus*, *G. tschenki*) have endotrophic tadpoles.

Given this major difference and the uncertain phylogenetic relationships of the two clades, we consider it most appropriate to propose a new subgenus for the species of the *Gephyromantis asper* clade.

***Asperomantis* subgen. n.**

ZooBank LSID: urn:lsid:zoobank.org:act:475FD179-D2D0-4208-B46C-68C25083CC18

Type species: *Rana aspera* Boulenger, 1882

Contained species: *Gephyromantis asper* (Boulenger, 1882), *G. ambohitra* (Vences & Glaw, 2001), *G. ceratophrys* (Ahl, 1929), *G. spinifer* (Blommers-Schlösser & Blanc, 1991), *G. tahotra* Glaw, Köhler & Vences, 2011, and *G.* sp. Ca28.

Diagnosis: Medium-sized terrestrial to scansorial rainforest frogs (adults 27–41 mm SVL). Characterized as belonging to the family Mantellidae by the presence of intercalary elements between terminal and subterminal phalanges of fingers and toes, femoral glands of type 2 (Glaw et al. 2000) in males, absence of nuptial pads, and apparent absence of release calls in males. Dorsum granular with a distinct pattern of longitudinal ridges, typically with inner and outer dorsolateral ridges as defined by Vences & Glaw (2001). Tarsal spines and heel spine typically present. Humeral gland present in males of at least some species. Tips of fingers and toes with moderately enlarged disks. Webbing present between toes, absent between fingers. Males with a blackish, paired, subgular vocal sac. Maxillary and vomerine teeth present; tongue bifid. Generalized exotrophic tadpoles developing in streams.

Most *Asperomantis* specimens can be easily distinguished from members of all other subgenera in the genus *Gephyromantis* and most other mantellid species by the presence of a (usually distinct) light spot in or near the centre of the tympanum, which is visible both in life (see Figs 8–13) and in preservative. Such a spot is usually absent in all other mantellids except *Laliostoma labrosum* and very few individuals of *Mantidactylus* (subgenus *Brygoomantis*) (see photos in Glaw & Vences 2007).

Etymology: The subgeneric name is a composite of the Latin word *asper*, meaning rough, and the Greek word *mantis*, meaning treefrog (see Vences et al. 1999 for the derivation of *mantis*). It refers to the rough granular dorsal skin shared by frogs of this clade, including its type species *G. asper*. The gender of this subgenus is masculine.

Species accounts

Gephyromantis asper (Boulenger, 1882)
(Fig. 8)

Name-bearing type: Lectotype of *Rana aspera* Boulenger, 1882, BMNH 1882.3.16.80, adult male, collected in "East Betsileo" by W. D. Cowan according to the original description. Lectotype designation by Vences & Glaw (2001).

Additional type material: Paralectotypes, BMNH 1882.3.16.81–90, from the same locality and collector as the lectotype.

Remark: This species has been referred to as *Gephyromantis* sp. Ca27 by Vieites et al. (2009) and Perl et al. (2014).

Distribution: Six localities are here assigned to this species (Fig. 2). For three of these, specimens can be assigned on

the basis of DNA sequences (see Fig. 1 and Supplementary Table 1 for voucher specimens): Mandraka (ca -18.929, 47.894, 1,152 m a.s.l.); Tsinjoarivo (Camp 2, -19.7165, 47.8216, 1,300 m a.s.l.; and Camp 3, -19.7199, 47.8570, 1,319 m a.s.l.), and Anjozorobe (-18.4214, 47.9383, 1,315 m a.s.l.). Three localities are assigned to the species on the basis of call recordings, or morphological examination of specimens: Andasibe (ca -18.93, 48.42, 920 m a.s.l.) based on a series of well-preserved specimens collected by R. Blommers-Schlösser (Supplementary Table 1); Ankeniheny (ca -19.17, 48.03, 1,000 m a.s.l.) based on our call recordings (Fig. 3); and Mantadia (ca -18.83, 48.47, 950–1,000 m a.s.l.) based on our call recordings as reported in Vences & Glaw (2001).

Numerous other localities have been assigned to this species historically (see Blommers-Schlössser & Blanc 1991, Vences & Glaw 2001, Glaw & Vences 2007, Glaw et al. 2011), but are not considered here given that the possible existence of other species requires confirmation by genetic or bioacoustic data.

Natural history: *Gephyromantis asper* is usually found in pristine or disturbed rainforest. Like other species of this group, it does not seem to dwell in secondary habitats. Specimens may be observed on the forest floor during the day, but males will call from perches 50–200 cm above the ground only at night (Blommers-Schlösser 1979, Vences & Glaw 2001).

Advertisement call: The calls of frogs assigned to *G. asper* all agree in being composed of short notes (5–26 ms) that are repeated in series with somewhat irregular intervals (Fig. 6). A note series may contain up to 70 notes. Within calls, three to six notes form a group separated from the following group by a slightly longer inter-note interval, i.e., two to five shorter inter-note intervals are followed by one longer interval. Within these note groups, the amplitude usually increases from the first to the last note. When listening, this temporal and energetic pattern brings to mind the sound of a galloping horse. This general pattern is evident in calls recorded at Mandraka and Ankeniheny, whereas mostly four to five notes are grouped in calls from Mandraka, compared to mostly three in a note group in calls from Ankeniheny (see Fig. 3). For numerical parameters of calls, see Table 2.

This galloping pattern is unique among the calls of all species in the *G. asper* group. Notes in calls of other species all are repeated at regular intervals. The note duration in *G. asper* calls is similar to those in calls of *G. ceratophrys*, but the intervals in the latter are much longer (Fig. 7). *Gephyromantis ambohitra* and *G. tahotra* both have calls with significantly longer notes.

Larval morphology: Tadpoles have not yet been described in detail, but Randrianiaina et al. (2011) published a photograph of the mouthparts of a tadpole from Mandraka. As with other *Gephyromantis*, the tadpoles are exotrophic with rather generalized mouthparts.

Gephyromantis ceratophrys (Ahl, 1929)
(Fig. 9)

Name-bearing type: Lectotype of *Mantidactylus ceratophrys* Ahl, 1929, ZMB 10443, adult female, collected in "Betsileo" by J. M. Hildebrandt according to the original description. Lectotype designation by Vences & Glaw (2001).

Additional type material: Paralectotypes, ZMB 10444, 50501 and 50502, three adult females, from the same locality and collector as the lectotype. According to the original description (Ahl 1929) there were six syntypes (see also Frost 2016), but we have been unable to locate two of these specimens in the collection of the Berlin museum. Hence, two additional paralectotypes for now are to be considered as lost.

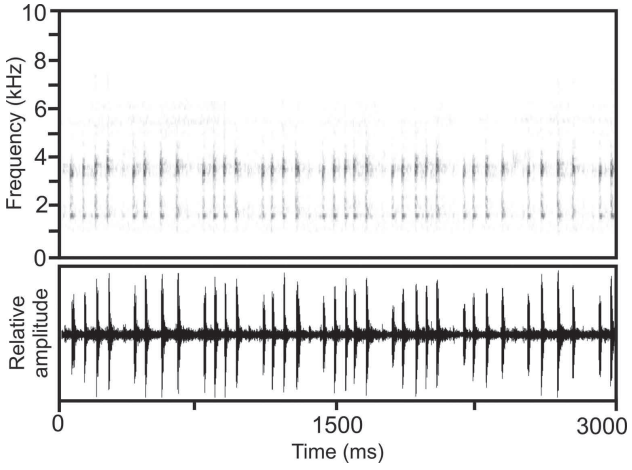

Figure 6. Audiospectrogram and oscillogram of a section of a call of *Gephyromantis asper* from Mandraka.

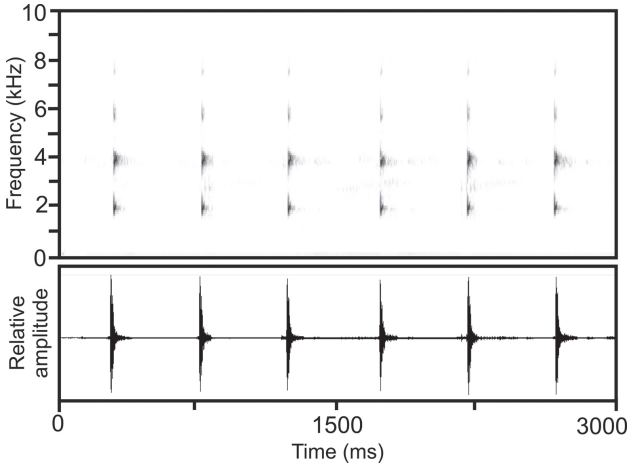

Figure 7. Audiospectrogram and oscillogram of a section of a call of *Gephyromantis ceratophrys* from Ranomafana.

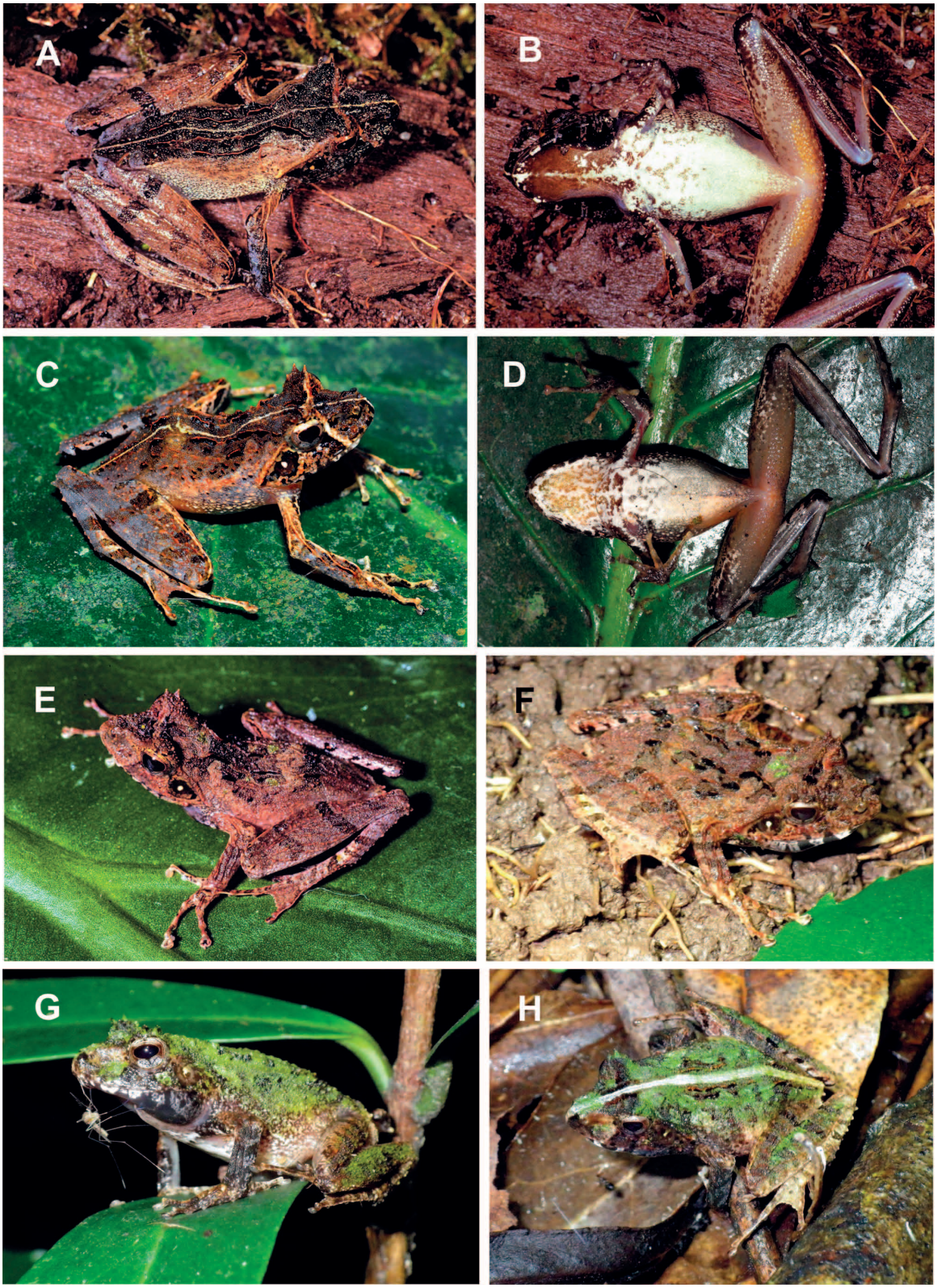

Figure 8. Specimens of *Gephyromantis asper* in life: (A, B) male from Ankeniheny (ZFMK 60789; call voucher) with weakly expressed femoral glands; (C, D) female from Tsinjoarivo (ZSM 299/2010); (E) male from Mandraka (ZSM 5046/2005); (F) male from Mandraka (ZSM 3221/2012); (G, H) males from Mandraka (not collected) with green dorsal coloration (note the mosquito sucking on the male depicted in G).

Figure 9. Specimens of *Gephyromantis ceratophrys* (all from Ranomafana) in life: (A, B) male (ZSM 1876/2008); (C, D) male (ZSM 655/2003); (E, F) female (ZSM 656/2003); (G) male (ZSM 1877/2008; call voucher); (H) male (UADBA uncatalogued).

Remark: This species has been referred to as *G. asper* by Vieites et al. (2009), Perl et al. (2014) and in older literature, and as *G.* sp. aff. *asper* by Kaffenberger et al. (2012).

Distribution: Material collected by ourselves that can reliably be referred to this species as defined here stems exclusively from the Ranomafana National Park. Specimens were found within the National Park (ZSM 655–656/2003, without specific coordinates), and at a site near the road Fianarantsoa-Ranomafana, very close to the park's main entrance (ZSM 1876–1877/2008; ca -21.258, 47.418, 1,200 m a.s.l.).

Redescription based on the male voucher ZSM 1877/2008 (ZCMV 8010): Adult male (Fig. 9G), in a good state of preservation. Snout–vent length 29.6 mm. For measurements, see Supplementary Table 1. Body slender; head slightly longer than wide, slightly wider than body; snout acuminate in dorsal view, truncate in lateral view; nostrils directed laterally, slightly protuberant, much nearer to tip of snout than to eye; canthus rostralis distinct, concave; loreal region concave; tympanum distinct, round, its diameter 78% of eye diameter; supratympanic fold moderately distinct, straight; tongue ovoid, distinctly bifid posteriorly; vomerine teeth indistinct, in two small rounded aggregations, positioned posterolaterally to choanae; choanae rounded. Dark dermal fold (the inflatable parts of the vocal sacs) running along each lower jaw from commissure of mouth to middle of lower jaw. Arms slender, subarticular tubercles single; outer metacarpal tubercle very poorly developed and inner metacarpal tubercle relatively well developed; fingers without webbing; relative length of fingers $1 < 2 \leq 4 < 3$; finger disks distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaching beyond snout tip when hindlimb is adpressed along body; lateral metatarsals separated by webbing; inner metatarsal tubercle distinct, outer metatarsal tubercle small but recognizable; webbing formula of foot according to Blommers-Schlösser (1979) 1(1), 2i(1.5), 2e(1), 3i(2), 3e(1), 4i(2.5), 4e(2.5), 5(0.5); relative toe length $1 < 2 < 3 \leq 5 < 4$. Toe discs distinctly enlarged. Skin dorsally granular; ridges bordering middorsal band distinctly elevated, starting approximately 1 mm behind eyes (starting off bifurcated and converging toward mid-dorsum) and gradually vanishing in posterior portion of back. Additional, less distinct and interrupted longitudinal ridges in posterior portion of back. Two distinct, blackish interocular ridges, bordering beige vertebral band; supraocular tubercles enlarged to form small dermal spines; short dermal tarsal spine. Ventral skin smooth on throat and limbs, slightly granular in posterior portion of belly. Femoral glands well delimited externally. Dorsal coloration after seven years in preservative brown with a broad beige vertebral band from snout tip to vent, narrowest between eyes and broadest on lower back. Dorsal ridges bordered with black. Each hindlimb with two distinct, broad, dark brown cross-bands extending onto thigh and shank, and two narrower and less distinct brown cross-bands. Dorsal colour of forelimbs brown with two distinct cross-bands on lower arm. Tympanic region dark brown with a small yet distinct white spot in the centre of the tympanum. Lips with alternating brown and grey flecks. Dorsal colour fading gradually to light ventral colour on flanks. Ground colour of ventral side cream-white with fine brown pigment on the anterior part of the throat, some indistinct brown markings on chest, and greyish posterior part of belly. Dermal folds of vocal sacs blackish. Femoral glands uniformly whitish without dark pigments. Ventral sides of limbs dirty cream-white on thighs, with brown and cream banding on shanks; ventral sides of tarsi and feet blackish. The colour in life was similar to that in preservative: Throat with a drawn-out brown spot anteriorly, chest and belly largely white with some darker marbling; femoral glands yellowish; iris light brown.

Natural history: Specimens were observed at a rainforest site near the main entrance of Ranomafana National Park on 26 January 2008. Calling males were perched on branches and leaves of the low vegetation, between 50 and 150 cm above the ground. A larger chorus of > 15 males was heard, all within a comparatively small area of an estimated 500–1,000 m^2.

Advertisement call: The advertisement call of *G. ceratophrys* from Ranomafana consists of a series of short notes (20–44 ms) showing amplitude modulation with the highest call energy being present at the beginning and decreasing towards the end of the note (Fig. 7). Note series may contain more than 40 notes separated by rather long intervals (428–912 ms). Although these notes were emitted at regular intervals, there is considerable variation in the intervals from one note series to another, as males appear to speed up note repetition when motivated. In any case, intervals between notes relative to note duration are the longest known in calls of the subgenus *Asperomantis*. For numerical call parameters, see Table 2.

In contrast to *G. ambohitra* and *G. tahotra*, the notes are much shorter in duration in *G. ceratophrys* (Table 2). Compared to calls of *G. asper* that consist of notes of similar length, the inter-note intervals are about eight times longer in calls of *G. ceratophrys* and follow a much more regular pattern.

Larval morphology: The tadpoles of this species have not yet been described in detail, but Randrianiaina et al. (2011; as *G.* sp. aff. *asper*) mention that they are exotrophic with rather generalized mouthparts.

Gephyromantis spinifer (Blommers-Schlösser & Blanc, 1991)
(Fig. 10)

Name-bearing type: Holotype of *Mantidactylus spiniferus* Blommers-Schlösser & Blanc, 1991, MNHN 1972.1450, adult male, from "Chaines Anosyennes" according to the original description, given as "Camp IV, Chaines Anosy-

ennes" in the MNHN catalogue, collected in November–December 1971 by C. P. Blanc. Camp IV (Ranomandry) was mapped by Paulian et al. (1973) who also provided its altitude (550 m). Based on this map we estimated the position of Camp IV in Google Earth as -24.137750, 47.073204, corresponding to an altitude of ca. 580 m a.s.l.). We here provisionally follow Dubois (1992) and Glaw & Vences (2007) in amending the species name *spiniferus* to *spinifer*, similar to the situation in *Gephyromantis plicifer* (originally *Rana plicifera* Boulenger, 1882). Final clarification of this nomenclatural issue remains pending.

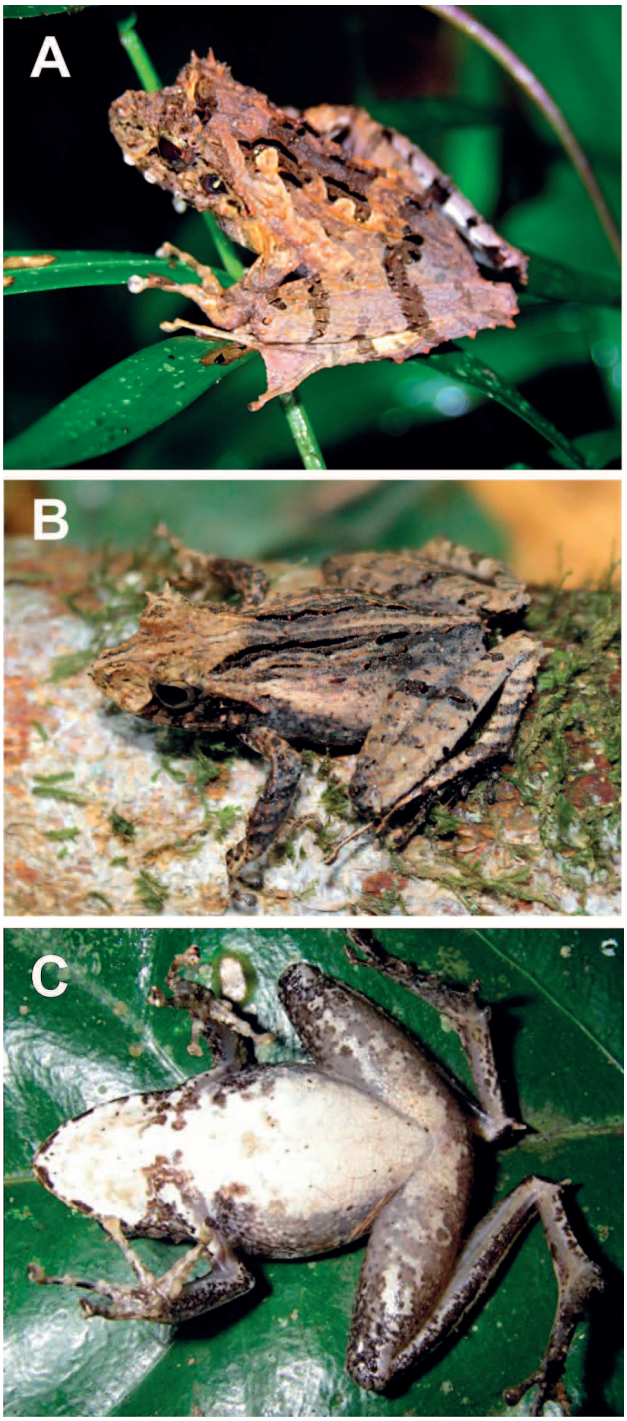

Figure 10. Specimens of *Gephyromantis spinifer* in life: (A) male from Beampingaratra near Andohahela; (B, C) female from Pic Ivohibe (ZSM 826/2014).

Additional type material: Paratypes MNHN 1972.1440 and 1972.1470, one adult female and one male, from the Chaînes Anosyennes Massif.

Distribution: Besides the type locality in the Anosy Massif (Camp IV, Chaînes Anosyennes) in southeastern Madagascar, and a nearby locality (Andohahela; photo by A. P. Raselimanana in Glaw & Vences 2007), our data now allow to confirm three further localities for which DNA sequences are available: (1) Andreoky / Beampingaratra near Andohahela (-24.4521, 46.8619, 1,049 m a.s.l.) based on two specimens collected on 20 May 2010 by F. M. Ratsoavina, (2) Befotaka-Midongy National Park, campsites Kilimagnarivo (-23.79750, 47.00961, 690–890 m a.s.l.) and Rozabe (-23.73658, 47.02303, 630–850 m a.s.l.) (Bora et al. 2007), and (3) Ivohibe (-22.48239, 46.95136, 949 m a.s.l.) based on a specimen collected on 8 November 2014 by A. Rakotoarison and M. C. Bletz. Nussbaum et al. (1999) reported the species from three rainforest sites in the Andohahela Reserve (site 1: -24.63194, 46.77555, 440 m a.s.l.; site 2: -24.6, 46.74166, 810 m a.s.l.; site 3: -24.58444, 46.73555, 1,200 m a.s.l.), with a total altitudinal range of 420–1250 m. Andreone & Randrianirina (2007) found *M. spinifer* at two sites in the Kalambatritra Special Reserve ("Befarara", -23.42361, 46.46361, 1450–1,700 m a.s.l. and "Befarafara", -23.3887, 46.49, 1,500–1,750 m a.s.l.). A discussion of older locality records was provided by Vences & Glaw (2001).

Natural history: At Beampingaratra, specimens were found in dense rainforest with a closed canopy (tree height about 15 m). The individual from Pic Ivohibe was encountered on the ground hopping through the leaf litter during the day. The habitat was rainforest in close proximity to a small stream. It was relatively intact there, but very close to a wide trail.

Advertisement call and larval morphology: Unknown.

Gephyromantis ambohitra (Vences & Glaw, 2001)
(Fig. 11)

Name-bearing type: Holotype of *Mantidactylus ambohitra* Vences & Glaw, 2001, ZSM 1084/2001 (originally ZFMK 57418), adult male, from Montagne d'Ambre, collected on 14–17 March 1994 by F. Glaw, N. Rabibisoa and O. Ramilison.

Additional type material: Paratypes MNHN 1893.244–245 (two females) from Montagne d'Ambre, MNHN 1893.246 (female), 1893.248 (male), 1893.249–250 (two females), 1893.252 (female), 1893.253 (juvenile), 1991.3148 (previ-

ously 1893.246A; female), all from Mararaomby (Montagne d'Ambre), sent to the Paris museum by Alluaud and Belly in 1893; MNHN 1972.573 (female) from Montagne d'Ambre; MNHN 1975.314 (female), 1975.322 (female), 1975.325 (female), 1975.329 (female), 1975.330–331 (two males) from Les Roussettes (Montagne d'Ambre), all without precise collecting data; MTKD 37424 (male) from Montagne d'Ambre; ZFMK 57419 (female) from Montagne d'Ambre, with same collecting data as holotype; and ZFMK 62204–62205 (two subadults), from Montagne d'Ambre, ca 1000 m a.s.l., collected on 26 November 1995 by J. Köhler and J. Steinbrecher.

Distribution: This species is known from three localities in northern Madagascar, all of which are confirmed by molecular data. (1) The type locality Montagne d'Ambre (ca -12.64, 49.16) where it is widespread and occurs within an altitudinal range of 900–1,250 m a.s.l. (Raxworthy & Nussbaum 1994, as *Mantidactylus asper*), (2) Campsite 1 at the Manongarivo Reserve (-13.97694, 48.42194, 751 m a.s.l.) where specimens were collected and calls recorded in February of 2003 by F. Glaw and M. Vences, (3) Mahalevona (Amparihy) at the Masoala Peninsula (-15.41783, 49.941, 667 m a.s.l.) based on a specimen (MRSN A2851) collected on 09 February 2002 by J. E. Randrianirina.

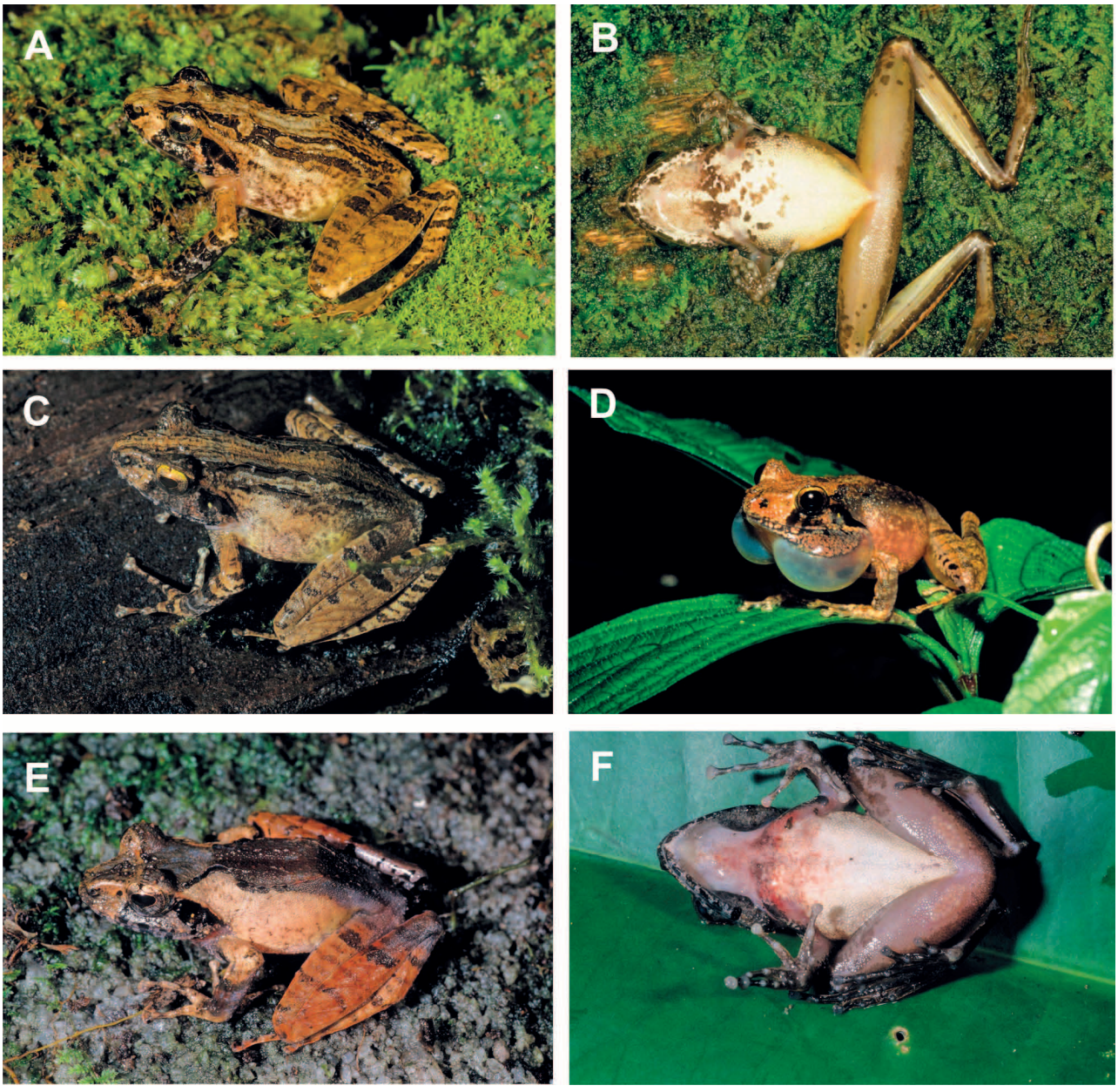

Figure 11. Specimens of *Gephyromantis ambohitra* in life: (A, B) female from Montagne d'Ambre; (C, D) males from Montagne d'Ambre; (E, F) male from Manongarivo.

Natural history: At Montagne d'Ambre, specimens were found on the forest floor during the day, and calling males were observed during rain at 20–22 h, calling from perches on bushes 60–150 cm above the ground at the edge of rainforest. At Manongarivo, males calling from perches at similar heights above the ground were recorded on 3 February 2003 at night.

The individual from Masoala was found during the night. It was encountered in rainforest (most probably a secondary regrown forest) at a site in close proximity to deforested areas.

Advertisement calls: Calls from two localities, Montagne d'Ambre and Manongarivo, have been described by Glaw et al. (2011). Their oscillograms, illustrating general call structure, are reproduced in Fig. 4, and temporal and spectral call variables are given in Table 2.

Larval morphology: Generalized and exotrophic tadpoles from the type locality, Montagne d'Ambre, have been described by Randrianiaina et al. (2007).

Gephyromantis tahotra Glaw, Köhler & Vences, 2011 (Fig. 12)

Name-bearing type: Holotype of *Gephyromantis tahotra* Glaw, Köhler & Vences, 2011, ZSM 193/2005 (field number FGZC 2882), adult male, from a campsite locally called "Camp Simpona", Marojejy National Park, northeastern Madagascar, collected on 16 February 2005 by F. Glaw, M. Vences and R. D. Randrianiaina.

Additional type material: Paratypes ZSM 191/2005 (FGZC 2815), and ZSM 192/2005 (FGZC 2848), two females, with same data as holotype, except the collection date of ZSM 192/2005 (17 February 2005).

Distribution: The species is known from five localities, all on the basis of specimens collected by us (Supplementary Table 1) and sequenced for mitochondrial DNA: (1) The type locality, Camp Simpona in the Marojejy Massif (-14.43665, 49.74335, 1,326 m a.s.l.), (2) the Tsaratanana Massif, at a campsite locally known as Matsabory Maiky (or Matsaborimaiky) (-14.15256, 48.95728, 2,021 m a.s.l.), (3) Ambodikakazo Forest south of the Tsaratanana Massif (-14.2131, 48.9052, 1,310 m a.s.l.), (4) Antsirakala forest fragment near Bemanevika (-14.43061, 48.60179, 1,466 m a.s.l.), and (5) a site in the Sorata Massif (-13.6851, 49.4417, 1,279 m a.s.l.).

Natural history: All specimens were found in pristine or slightly disturbed rainforest. Calling males at Marojejy were observed perched on leaves about 50 cm above the ground (Glaw et al. 2011). Calls at Sorata were heard at night from 26 November through 1 December 2012. A calling male (ZSM 1550/2012) had a distinctly bilobate vocal sac and was calling from a fern leaf ca 50 cm above the ground. Specimens from Tsaratanana, Ambodikakazo and Antsirakala were found on the forest floor during the day.

Advertisement call: Calls of *G. tahotra* have been described from Marojejy by Glaw et al. (2011). Calls from Sorata were recorded on 1 December 2012 at 22:10 h (call voucher ZSM 1550/2012, temperature unknown) in an aggregation of several calling males sitting around a dry cavity, several metres from a small stream. In this aggregation, two call types were recorded: (1) A call (Fig. 4) consisting of 5–6 pulsatile notes of 98–120 ms in duration that were repeated at regular intervals (143–172 ms). These calls were classified as the advertisement call as they were emitted exclusively without obvious male–male interaction. This call is very similar to those from Marojejy with overlaps in all numerical parameters except a slightly shorter note duration and a slightly higher pulse rate within notes (360 pulses/second) (Tab. 2). (2) A call emitted within a chorus of several males and not heard in isolated males, is here classified as call type 2 (Fig. 4). This call consisted of distinctly shorter notes of 61–71 ms (67±3) separated by much shorter intervals of 70–80 ms (74±3; n=26), whereas the frequency is within the same range as in the advertisement call. The note series of call type 2 contained up to 30 notes. Another difference is obvious in the amplitude modulation of the notes, with the lowest energy being present in the middle of the note (Fig. 4).

Larval morphology: Tadpoles have not yet been described in detail, but Randrianiaina et al. (2011; *G.* sp. aff. *ambohitra*) mention that they are exotrophic with rather generalized mouthparts.

Gephyromantis sp. Ca28 (Fig. 13)

Referred material: Two specimens, ZSM 1731/2010 (field number ZCMV 12303) from a forest near a site locally named Antsahan'i Ledy at the base of the Tsaratanana Massif, collected on 9 June 2010; and ZSM 1734/2010 (ZCMV 12605) from a forest fragment between Bealanana-Antsohihy, collected on 29 June 2010, both by M. Vences, D. R. Vieites, R. D. Randrianiaina, F. M. Ratsoavina, S. Rasamison, A. Rakotoarison, F. Rasoloarison, E. Rajeriarison, and T. Rajoafiarison.

Identity: This candidate species was first identified by Perl et al. (2014) based on divergent sequences in its mitochondrial COI gene. We here confirm its distinctness on the basis of the mitochondrial 16S rRNA and the nuclear Rag1 gene (Fig. 1). However, one specimen of *G. tahotra* from the same region south of the Tsaratanana Massif had a Rag1 haplotype very similar to that of this candidate species, suggesting possible gene flow or incomplete lineage sorting of nucDNA between these forms. More fieldwork is necessary to ascertain the taxonomic status and distribution of this candidate species.

Distribution: The species is known from two localities: (1) A forest at the base of the Tsaratanana Massif near the Antsahan'i Ledy campsite, (-14.23319, 48.98001, 1,207 m a.s.l.), and (2) a forest fragment between Bealanana and Antsohihy (-14.72145, 48.56272, 1,187 m a.s.l.).

Natural history: Specimens were found within disturbed rainforest.

Advertisement call and larval morphology: Unknown.

Figure 12. Specimens of *Gephyromantis tahotra* in life: (A, B) male from Sorata (ZSM 1544/2012); (C, D) female from Tsaratanana (ZSM 1732/2010); (E) female from Tsaratanana (ZSM 529/2014); (F) female from Tsaratanana (ZSM 560/2014).

Discussion

This study presents an updated account of the distribution of species and candidate species in the *Gephyromantis asper* clade, here proposed as a new subgenus *Asperomantis*, and documents our arguments to resurrect *G. ceratophrys* as the name for the population from the Ranomafana National Park formerly regarded as representing *G. asper*. Deciphering the phylogenetic relationships of these frogs was not the main purpose of the present paper. Yet, when revising their species-level taxonomy, we also revisited the monophyly of *Duboimantis*, which had already been questioned in recent studies (e.g., Vieites et al. 2009, Wollenberg et al. 2011, Pyron & Wiens 2011, Kaffenberger et al. 2012, Perl et al. 2014), together with the identification of a lack of monophyly of the subgenus *Duboimantis* in which they have been classified (Glaw & Vences 2006). The placement of the *G. asper* clade in our new subgenus *Asperomantis* is in agreement with the taxon-naming criteria suggested by Vences et al. (2013). As the phylogenetic placement of *Asperomantis* remains uncertain, we cannot exclude that it may eventually turn out to be a sister group to *Duboimantis*, but keeping all species in *Duboimantis* is a clearly worse alternative under the clade stability criterion (Vences et al. 2013). *Asperomantis* is supported as monophyletic, and its recognition will render *Duboimantis* monophyletic (Kaffenberger et al. 2012).

Most specimens of *Asperomantis* are easily distinguished from *Duboimantis* and other mantellids by a light spot (of unknown significance) on the tympanum, which is only rarely indistinct. Furthermore, larval ecomorphology (exotrophic vs endotrophic) along with the combination of several characters of adult morphology (inner and outer dorsolateral ridges, heel spines, paired subgular vocal sacs) also allow for their diagnosis.

According to Kaffenberger et al. (2012), *G. ambohitra* is the sister taxon of all other *Asperomantis* species (except for *G.* sp. Ca28, which was not included in that study). According to our study, the next species to split was *G. tahotra* as the sister taxon of *G.* sp. Ca28 + *G. ceratophrys* + *G. asper* + *G. spinifer*. The basal positions of the northern Malagasy species suggest an origin of this subgenus in this region.

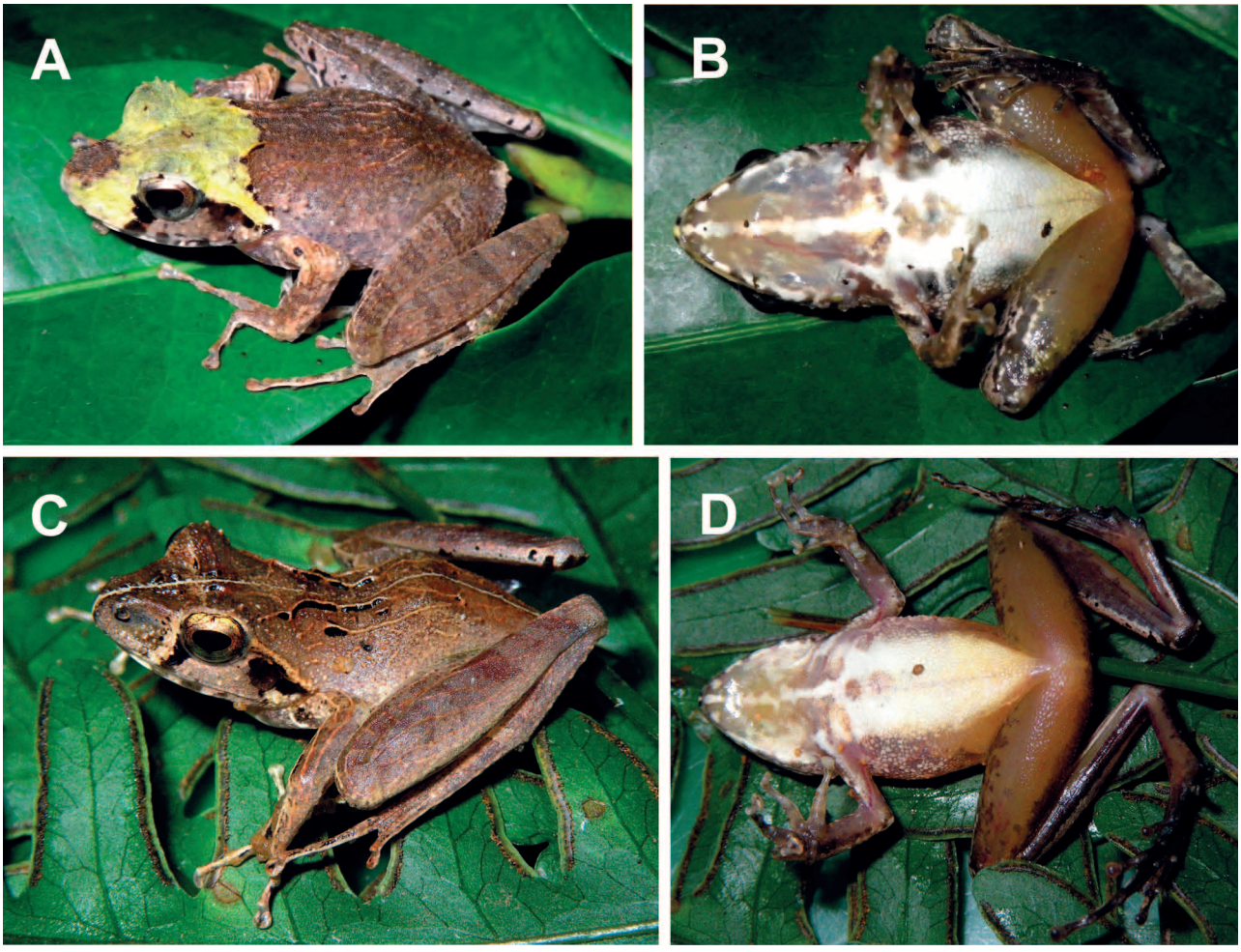

Figure 13. Specimens of *Gephyromantis* sp. Ca28 from Bealanana, Tsaratanana in life: (A, B) subadult male (ZSM 1731/2010); (C, D) female (ZSM 1734/2010).

Our study confirms the position of *G. ambohitra* as the sister to all other *Asperomantis* based on mtDNA (Fig. 1A), but relatively low differences in Rag1 suggest similarities between *G. ambohitra* and *G. tahotra* (Fig. 1B). The complete lack of Rag1 haplotype-sharing between the species and candidate species recognized herein suggests that gene flow between them is absent or exceedingly rare, especially when considering that three taxa (*G. ambohitra, G. tahotra, G.* sp. Ca28) co-occur in northern Madagascar. However, the similarity of one *G. tahotra* Rag1 haplotype with the haplotye of *G.* sp. Ca28, and the similarity of two *G. ceratophrys* haplotypes with those of *G. tahotra*, may be seen as indications of incomplete lineage sorting.

In general, it would appear that species of *Asperomantis* are restricted to higher altitudes, typically above 1,000 m a.s.l. One species that appears to be adapted to somewhat lower situations is *G. ambohitra*: at its type locality Montagne d'Ambre it occurs between 900 and 1,250 m a.s.l. (Raxworthy & Nussbaum 1994, as *Mantidactylus asper*), but it has been recorded from as low as 750 m a.s.l. in Manongarivo and at 667 m a.s.l. in Masoala. *G. spinifer* likewise appears to descend to lower altitudes of between 420–890 m a.s.l. at Andohahela and Befotaka-Midongy (Nussbaum et al. 1999, Bora et al. 2007), and *G. asper* is known from about 900 m a.s.l. around Andasibe and Mantadia. The three species occurring in northern Madagascar appear to have different vertical ranges: *G. ambohitra,* 750–1,250 m a.s.l.; *G.* sp. Ca28, 1,190–1,210 m a.s.l.; and *G. tahotra,* 1,280–2,020 m a.s.l. So far, no syntopic occurrence of any two species of *Asperomantis* has been recorded, but it is likely that the three species will be found together at sites around 1,200 m a.s.l. in northern Madagascar where their putative altitudinal preferences overlap. Along the east coast, contact zones are to be expected to exist between *G. asper* and *G. ceratophrys*, and between *G. ceratophrys* and *G. spinifer.*

All of these species appear to require rainforest for their survival and have never been found in young secondary vegetation or heavily degraded forest. According to the Global Amphibian Assessment for Madagascar (Andreone et al. 2005, 2008), *G. asper* is classified as Least Concern (LC), *G. ambohitra, G. spinifer*, and *G. tahotra* as Vulnerable (VU). The data presented in this paper redefine *G. asper*, revalidate *G. ceratophrys*, provide evidence for range extensions of *G. ambohitra* and *G. tahotra*, and confirm the previously assumed range of *G. spinifer.* This might mean that the assumptions employed for previous IUCN assessments are no longer valid and should be revised in the context of an ongoing new conservation assessment of Madagascar's frogs.

Gephyromantis ceratophrys, resurrected from synonymy herein, has not been thoroughly evaluated to date. It is currently known only from the Ranomafana National Park, although its distribution range probably extends farther north and towards the range of *G. asper*, and south towards the range of *G. spinifer.* Statuses of Endangered (EN) have been proposed for other anuran species known only from the Ranomafana National Park, such as *Anodonthyla emilei* and *A. moramora* (Vences et al. 2010). These assessments were based on the IUCN criteria (IUCN 2001) of an Extent of Occurrence of less than 5,000 km², all individuals being present in fewer than five locations, and a probably continuing decline in the extent and quality of much of their habitat. Given the current knowledge, it may be adequate to apply this same rationale also to *G. ceratophrys* for consistency, and classify this species as EN, considering however that this status might require modification if the species is found at other locations and thus demonstrated to have a wider Extent of Occurrence.

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Supplementary material

Supplementary Table S1. Morphometric measurements of the studied specimens of the subgenus *Asperomantis*.

Supplementary Table S2. Size of femoral glands in male specimens of the subgenus *Asperomantis*.