Article

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Molecular systematics of the subgenus *Gephyromantis* (*Phylacomantis*) with description of a new subspecies

MARK D. SCHERZ[1], JANINA RUDOLPH[2], MARIUS RAKOTONDRATSIMA[3], FANOMEZANA M. RATSOAVINA[4], ANGELICA CROTTINI[5,6,7], FRANCO ANDREONE[8], FRANK GLAW[9] & MIGUEL VENCES[2,*]

[1]*Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, 2100, Copenhagen Ø, Denmark*
✉ *mark.scherz@gmail.com;* ⬤ *https://orcid.org/0000-0002-4613-7761*
[2]*Zoologisches Institut, Technische Universität Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany*
✉ *janina.rudolph@tu-braunschweig.de;* ⬤ *https://orcid.org/0009-0003-5028-6932*
✉ *m.vences@tu-braunschweig.de;* ⬤ *https://orcid.org/0000-0003-0747-0817*
[3]*The Peregrine Fund's Project in Madagascar, B. P. 4113, Antananarivo 101, Madagascar*
✉ *mariusphr@yahoo.com;* ⬤ *https://orcid.org/0009-0004-2550-8326*
[4]*Mention Zoologie et Biodiversité Animale, Université d'Antananarivo, BP 906, Antananarivo 101, Madagascar*
✉ *fanomezanarts@gmail.com;* ⬤ *https://orcid.org/0000-0003-1661-1669*
[5]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal*
✉ *acrottini@cibio.up.pt;* ⬤ *https://orcid.org/0000-0002-8505-3050*
[6]*Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal*
[7]*BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal*
[8]*Museo Regionale di Scienze Naturali, Via G. Giolitti, 36, 10123 Torino, Italy*
✉ *franco.andreone@regione.piemonte.it;* ⬤ *https://orcid.org/0000-0001-9809-5818*
[9]*Zoologische Staatssammlung München (ZSM-SNSB), Münchhausenstr. 21, 81247 München, Germany*
✉ *glaw@snsb.de;* ⬤ *https://orcid.org/0000-0003-4072-8111*
*Corresponding author: ✉ *m.vences@tu-braunschweig.de*

Abstract

The mantellid subgenus *Phylacomantis* (genus *Gephyromantis*) currently contains four species of frogs distributed in the South (*G. corvus*, *G. kintana*), West (*G. atsingy*), North East, North, and Sambirano regions (*G. pseudoasper*) in Madagascar. We assess the molecular systematics of these amphibians based on extended sampling and analysis of multiple nuclear-encoded and mitochondrial genes. We confirm the delimitation of the four known species, which form monophyletic groups in a mitochondrial tree and separate phylogroups without haplotype sharing in haplotype networks of four nuclear markers. Newly included samples of *G. pseudoasper* from the North and North East regions confirm a comparatively low genetic variation of this species across its range, with pairwise genetic distance in the 16S gene not exceeding 1.4%. On the contrary, newly collected specimens from three sites in the North East and North West of the island strongly differed from all other *Phylacomantis*, with genetic distances exceeding 4.8% for the16S gene. Mitochondrial and nuclear markers strongly suggest them to be sister to *G. corvus* from the South. Due to haplotype sharing in two nuclear genes, absence of detectable morphological differences, and lack of data on bioacoustic differentiation, we describe these populations as a new subspecies, *G. corvus bakilana* **ssp. nov.**, emphasizing that this taxon may be elevated to species status in the future pending new data. This biogeographic pattern, with apparently disjunct sister lineages in the South vs. North West of Madagascar, is rare among Malagasy anurans and calls for increased exploration of the remaining humid forest fragments in the North West and West of the island.

Key words: Amphibia, Mantellidae, subspecies, *G. corvus bakilana* **ssp. nov.**

Introduction

Among the Madagascar-Comoro endemic frog family Mantellidae, *Gephyromantis* ranks third in species richness, with currently 57 species (Frost 2024) classified in six subgenera (Glaw & Vences 2006; Kaffenberger *et al.* 2012;

Vences *et al.* 2017). Most *Gephyromantis* are restricted to the humid forested regions of eastern and, especially, northern Madagascar, and are characterized by either generalized tadpoles developing in streams (subgenus *Asperomantis*) or non-feeding tadpoles (subgenera *Duboimantis*, *Gephyromantis*, *Laurentomantis*, and *Vatomantis*). An exception is the nocturnal, scansorial to terrestrial subgenus *Phylacomantis*. Three out of the four currently known species of *Phylacomantis* are distributed in comparatively humid relict forests in the South and West of Madagascar (geographic regions as defined in Boumans *et al.* 2007), and have specialized carnivorous tadpoles known to produce sounds (Randrianiaina *et al.* 2007; Reeve *et al.* 2011).

The taxonomic history of *Phylacomantis* has been characterized by some confusion due to the high morphological similarity of all four species, but at present, the four species and their geographic range are well delimited (Cocca *et al.* 2018, 2020): *G. corvus* and *G. kintana* co-occur in the Isalo Massif in the South; *G. corvus* is also known from forest fragments close to the Andringitra Massif (Anja, Sakaviro, Traranoro) and possibly from the Makay massif (Rakotondravony & Goodman 2011); *G. atsingy* appears to be restricted to the Tsingy de Bemaraha Massif, probably including Beanka in the West (Crottini *et al.* 2011; Raselimanana 2013); and *G. pseudoasper* is more widespread in the humid, transitional and dry forests of the North East, North and Sambirano regions (Glaw & Vences 2007).

During field work in 2009 and 2015/2016, we collected specimens of *Gephyromantis* clearly assignable to the subgenus *Phylacomantis* at three sites in the northern and eastern portions of the Sofia Region of Madagascar and previously assigned the candidate species number *G.* sp. Ca31 (Scherz *et al.* 2017). We here report on the identity of these frogs which, based on molecular phylogenetics, we find to be related to *G. corvus* from the South.

Materials and Methods

Material

This study is based on voucher specimens and tissue samples collected during field expeditions in Madagascar between 2000–2016. Specimens were caught during nocturnal and diurnal searches, either opportunistically by catching animals on the ground around swamps or in canyons, or by locating calling males. Vouchers were anesthetized by immersion in MS222 or chlorobutanol solution, and subsequently euthanized with an overdose of the same substances. After excision of tissue samples for molecular analysis—typically parts of thigh muscles stored in 1.5 ml vials with absolute ethanol—voucher specimens were fixed in 95% ethanol and subsequently transferred to 70% ethanol for long-term storage in the following collections: Museo Regionale di Scienze Naturali, Torino, Italy (MRSN), Université d'Antananarivo, Mention Zoologie et Biodiversité Animale, (UADBA), and Zoologische Staatssammlung München (ZSM). We use ACP or ACZCV, DRV, MSZC, FAZC, FGZC, and FGMV or ZCMV, to refer to extraction number and field numbers of A. Crottini, D. R. Vieites, M. D. Scherz, F. Andreone, F. Glaw, and M. Vences, respectively. A full list of all field numbers and museum catalogue numbers, as well as sequences and sequence accession numbers, is available as Supplementary Table 1 as Excel, and tab-delimited table from the Zenodo repository under https://doi.org/10.5281/zenodo.10840032. In addition, several sequences are from unvouchered tissue samples, held in the collection of A. Crottini (ACP series).

Morphometrics

Morphometric measurements were taken by MV using a manual caliper at an accuracy of 0.1 mm, as follows: snout–vent length (SVL); maximum head width (HW); head length from posterior edge of mouth opening in a diagonal line to tip of snout (HL); horizontal tympanum diameter (HTD); horizontal eye diameter (HED); distance between anterior edge of eye and nostril (END); distance between nostril and tip of snout (NSD); distance between both nostrils (NND); forelimb length, from limb insertion to tip of longest finger (FORL); hand length, from the hand insertion to the tip of the longest finger (HAL); hind limb length, from the cloaca to the tip of the longest toe (HIL); foot length excluding tarsus (FOL); foot length including tarsus (FOTL); tibia length (TIBL); femoral gland length (FGL); and femoral gland width (FGW). Webbing formula follows Blommers-Schlösser (1979). Geographical regions within Madagascar are named according to Boumans *et al.* (2007) and Brown *et al.* (2014, 2016); in addition, to more specifically characterize the extent of occurrence of the new lineage described herein, we specify that its range is within the Sofia Region, an administrative unit within Madagascar.

TABLE 1. Pairwise genetic distances (in %) calculated from a trimmed alignment of 510 nucleotides of a fragment of the mitochondrial 16S rRNA gene among species and subspecies of *Gephyromantis* (*Phylacomantis*). Values are given as mean, with minimum and maximum in parentheses, for all pairwise comparisons among included sequences of the respective taxa. Distances between *G. c. corvus* and *G. c. bakilana* **ssp. nov.** are highlighted in bold.

	G. atsingy	*G. c. corvus*	*G. kintana*	*G. pseudoasper*	*G. c. bakilana* **ssp. nov.**
G. atsingy	0.5 (0.0–1.0)				
G. c. corvus	11.6 (11.0–12.0)	0.5 (0.0–1.6)			
G. kintana	10.0 (9.8–10.4)	9.3 (8.8–9.7)	0.1 (0.0–0.6)		
G. pseudoasper	10.6 (10.0–14.3)	13.2 (12.2–15.9)	12.5 (12.0–15.5)	0.7 (0.0–1.4)	
G. c. bakilana **ssp. nov.**	11.4 (11.0–11.8)	**5.2 (4.8–5.3)**	9.5 (9.3–9.7)	12.8 (12.2–14.3)	0.1 (0.0–0.2)

Genetics

We extracted DNA from tissue samples using a standard salt extraction protocol (Bruford *et al.* 1992). We DNA barcoded previously unstudied *Phylacomantis* specimens for a fragment of the mitochondrial 16S rRNA gene (16S) that spans about half of the gene at its 3' terminal portion and that has regularly been used for molecular taxonomy of Malagasy amphibians (e.g., Vieites *et al.* 2009), using PCR-amplification with primers 16SAL (5'–CGCCTGTTTATCAAAAACAT–3') and 16SBH-new: (5'–CCTGGATTACTCCGGTCTGA–3'), modified from Palumbi *et al.* (1991), with the following cycling protocol: 94°C (90s), 33 x [94°C (45s), 55°C (45s), 72°C (90s)], 72°C (300s). The dataset was complemented with sequences available from GenBank (see https://doi.org/10.5281/zenodo.10840032). We furthermore sequenced a subset of samples for a fragment of the following four nuclear-encoded genes: (i) the recombination activating gene 1 (RAG-1), using primers Gephlut-RAG1-F1 (5'–ATGGAGAGCCAACCCCTATC–3') and Gephlut-RAG1-R1 (5'–KCCAGACTCGTTTCCTTCRC–3') with cycling protocol: 94°C (120s), 39 x [94°C (20s), 54°C (50s), 72°C (180s)], 72°C (600s) and using the sequencing primer RAG1-Manti-Seq1 (5'–GCAAAGCCVTTTATTGAAACC–3'); (ii) a fragment of the POMC gene with primers POMC-DRVF1 (5'–ATATGTCATGASCCAYTTYCGCTGGAA–3') and POMC-DRVR1 (5'–GGCRTTYTTGAAWAGAGTCATTAGWGG–3') of Vieites *et al.* (2007) with cycling protocol: 95°C (120s), 9 x [95°C (120s), 62°C (50s) - 1°C per cycle, 72°C (80s)], 30 x [95°C (120s, 52°C (50s), 72°C (80s)], 72°C (600s); (iii) a fragment of sacsin (SACS) using a nested approach (Shen *et al.* 2012) using external primers SACS-F2 (5'–AAYATHACNAAYGCNTGYTAYAA–3') and SACS-R2 (5'–GCRAARTGNCCRTTNACRTGRAA–3') and internal primers SACS-NF2 (5'–TGYTAYAAYGAYTGYCCNTGGAT–3') and SACS-NR2 (5'–CKGTGRGGYTTYTTRTARTTRTG–3') and with cycling protocol for both PCRs: 94°C (240s), 45 x [94°C (45s), 45°C (40s), 72°C (120s)], 72°C (600s); and (iv) a fragment of KIAA1239 using the same approach and cycling protocol of SACS, with external primers KIAA1239-F1 (5'–CARCCTTGGGTNTTYCA–3'), KIAA1239-R1 (5'–CMACAAAYTGGTCRTTR–3'), and internal primers KIAA1239-NF1 (5'–GAGCCNGAYATHTTYTTYG–3') and KIAA1239-NR1 (5'–TTCACRAANCCMCCNG–3') (Shen *et al.* 2012). Furthermore, for the purpose of a multigene phylogeny, we also complemented the mitochondrial data set of Kaffenberger *et al.* (2012), by sequencing fragments of the 12S rRNA, cytochrome *b* (COB) and cytochrome oxidase subunit I (COX1) genes of the newly discovered north-western lineage of *Phylacomantis*, using primers and cycling protocols given therein.

PCR products were purified with Exonuclease I and Shrimp Alkaline Phosphatase digestion and sent for sequencing on automated DNA sequencers to LGC Genomics (Berlin). Chromatograms were checked and corrected for obvious errors with CodonCode Aligner 3.7.1 (Codon Code Corporation, Dedham, MA, USA). All newly obtained sequences were submitted to GenBank and are available under the following accession numbers: PP457120–PP457253 and PP460911–PP460947.

Sequences were aligned using the MAFFT algorithm (Katoh & Standley 2013) and (for the multigene dataset) concatenated in Concatenator (Vences *et al.* 2022), which is part of the iTaxoTools toolkit (Vences *et al.* 2021). For the 16S data set, we inferred the best fitting substitution model under the Bayesian Information Criterion in MEGA7 (Kumar *et al.* 2016), and inferred a phylogeny under the Maximum Likelihood (ML) criterion in RAxML (Stamatakis 2014), assessing node support with 500 full ("thorough") bootstrap replicates. For the multigene tree, we inferred (i) a ML tree based on the unpartitioned data set with RAxML as with the 16S data set, and a further ML

tree from a partitioned analysis with IQ-TREE v.2.1.2 (Minh *et al.* 2020), with a per-gene partition with independent substitution models selected by the Bayesian Information Criterion in ModelFinder (Kalyaanamoorthy *et al.* 2017), and 1000 ultrafast bootstrap replicates. Trees were initially visualized in Figtree v1.4.4 (Rambaut 2018), and subsequently graphically edited in Corel Draw X3 (Alludo, Ottawa, Canada).

To graphically represent the relationships among alleles (haplotypes) of the nuclear-encoded gene fragments, we used a network approach. Alleles (haplotypes) were inferred with the PHASE algorithm (Stephens *et al.* 2001) and a Fitch tree genealogy (≈network) was reconstructed in Hapsolutely software, which is part of the iTaxoTools toolkit (Vences *et al.* 2021), following the methodological approach of Salzburger *et al.* (2011).

Pairwise genetic distances were calculated from the 16S alignment using TaxI2 (Vences *et al.* 2021), including the hypervariable region (and coding indels as missing data; the alignment included only few indels, especially one poly-C stretch in one loop region with different lengths in different *G. pseudoasper*; see https://doi.org/10.5281/zenodo.10840032 for the alignment used). Species partitions were inferred using ASAP (Puillandre *et al.* 2021) and subsequently we compared the favored ASAP partition for concordance with evidence from differentiation in the nuclear-encoded genes. Molecular diagnostic sites were determined with MolD (Fedosov *et al.* 2022) and are given relative to the full 16S sequence of *G. pseudoasper* (GenBank accession AB325880).

Results

Molecular genetic and morphological differentiation

The ML tree based on 127 ingroup sequences of the subgenus *Gephyromantis* (*Phylacomantis*) for a 540-nucleotide alignment of a fragment of the mitochondrial 16S gene (Fig. 1) resulted in four main clades corresponding to the nominal species of subgenus *Gephyromantis* (*Phylacomantis*), with very limited variation uncovered within *G. atsingy*, *G. kintana*, and *G. pseudoasper* (Table 1). Samples from across the range of *G. pseudoasper*, including newly sequenced specimens from Montagne d'Ambre and Marojejy (in the North East and North regions; Fig. 2) clustered closely with other sequences attributed to this species. Within *G. corvus*, two distinct subclades were recovered, one of which corresponded to new samples from the Sofia administrative region (spanning North East and North West biogeographic regions). *Gephyromantis corvus* and *G. kintana* were placed as sister taxa.

ASAP suggested a species partition with lowest ASAP score (1.5) containing five ingroup subsets corresponding to the four nominal species in *Gephyromantis* (*Phylacomantis*), plus the newly discovered lineage from the Sofia Region as fifth subset. The second best species partition (ASAP score 2.0) included these populations within *G. corvus*.

Genetic distances in the mitochondrial 16S gene were very high between nominal species of *Gephyromantis* (*Phylacomantis*) (8.8–15.9% uncorrected pairwise distance; Table 1) and low within these species (up to a maximum of 1.6% within *G. corvus* from the South). The populations from the Sofia Region differed by 4.8–5.3% from *G. corvus* populations in the South, and by 9.3–14.3% from the other species.

Haplotype genealogies reconstructed from phased nuclear-encoded genes split all four nominal species into separate phylogroups without haplotype sharing among them (Fig. 3). The sequences of the frogs resembling *G. corvus* obtained from the Sofia Region populations were in all cases grouped close to sequences of *G. corvus* from Isalo in the South, with haplotype sharing in two genes (RAG-1, KIAA1239) and absence of haplotype sharing in the other two genes (POMC, SACS).

A multigene phylogeny (Fig. 4) of several mitochondrial and nuclear-encoded gene fragments confirmed the phylogenetic relationships suggested by the single 16S fragment ML phylogeny, placing a sample from the Sofia Region (Makira) as sister to *G. corvus* from Isalo with high support, and resolving *G. corvus* (NW+SW) and *G. kintana* as sister groups.

Morphologically, the specimens from the Sofia Region did not reveal any conspicuous features distinguishing them from *G. corvus* from the South; they share with that species blackish vocal sacs, tubercular dorsal skin, dark belly, and body size.
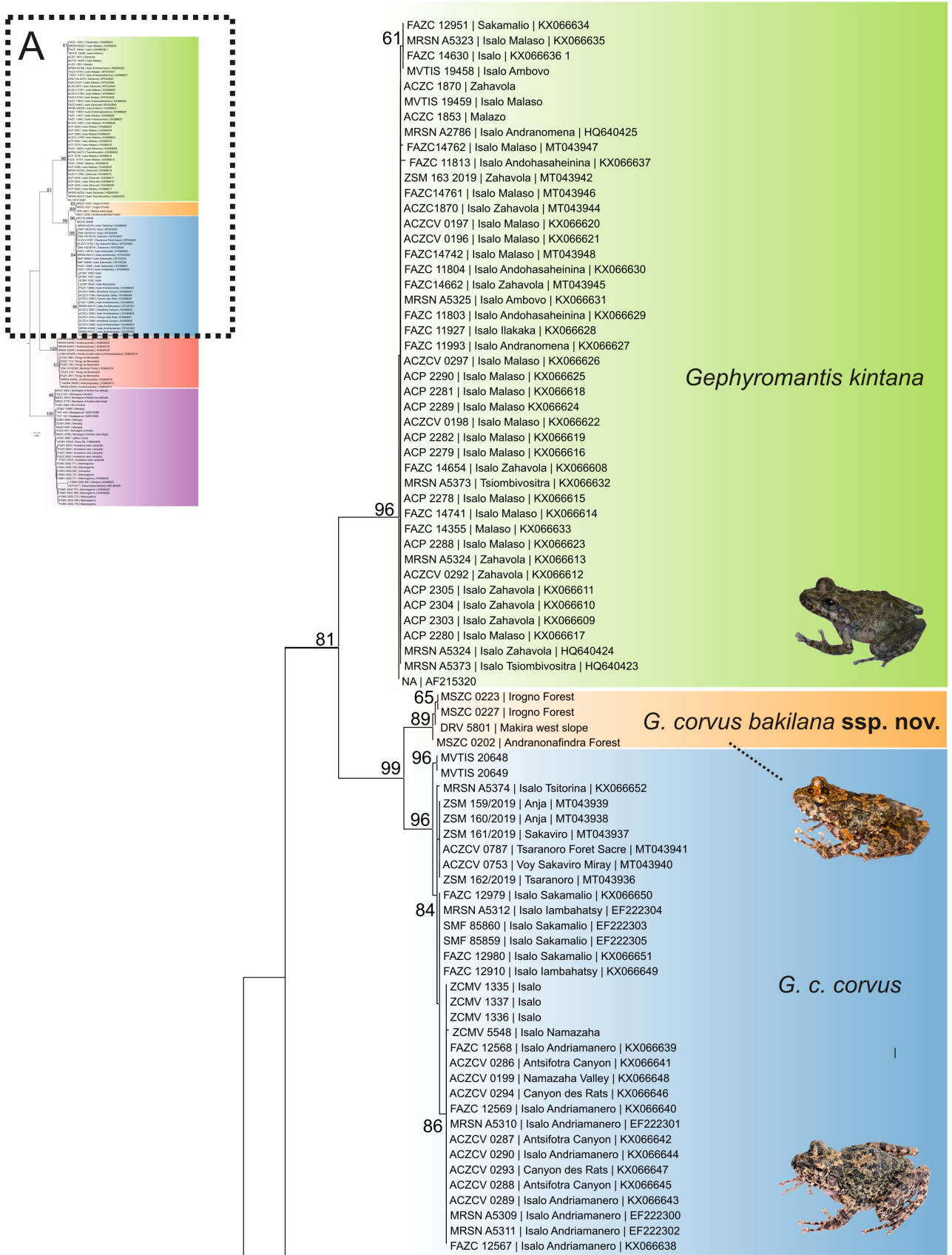
FIGURE 1. Maximum likelihood tree based the mitochondrial 16S rRNA gene (540 nucleotides, 127 samples) of the subgenus *Phylacomantis*. The tree was rooted with sequences of *Gephyromantis ambohitra* (subgenus *Asperomantis*; not shown). Numbers at nodes are bootstrap proportions in percentage (only shown for values >50%, and not shown for shallow intraspecific nodes).
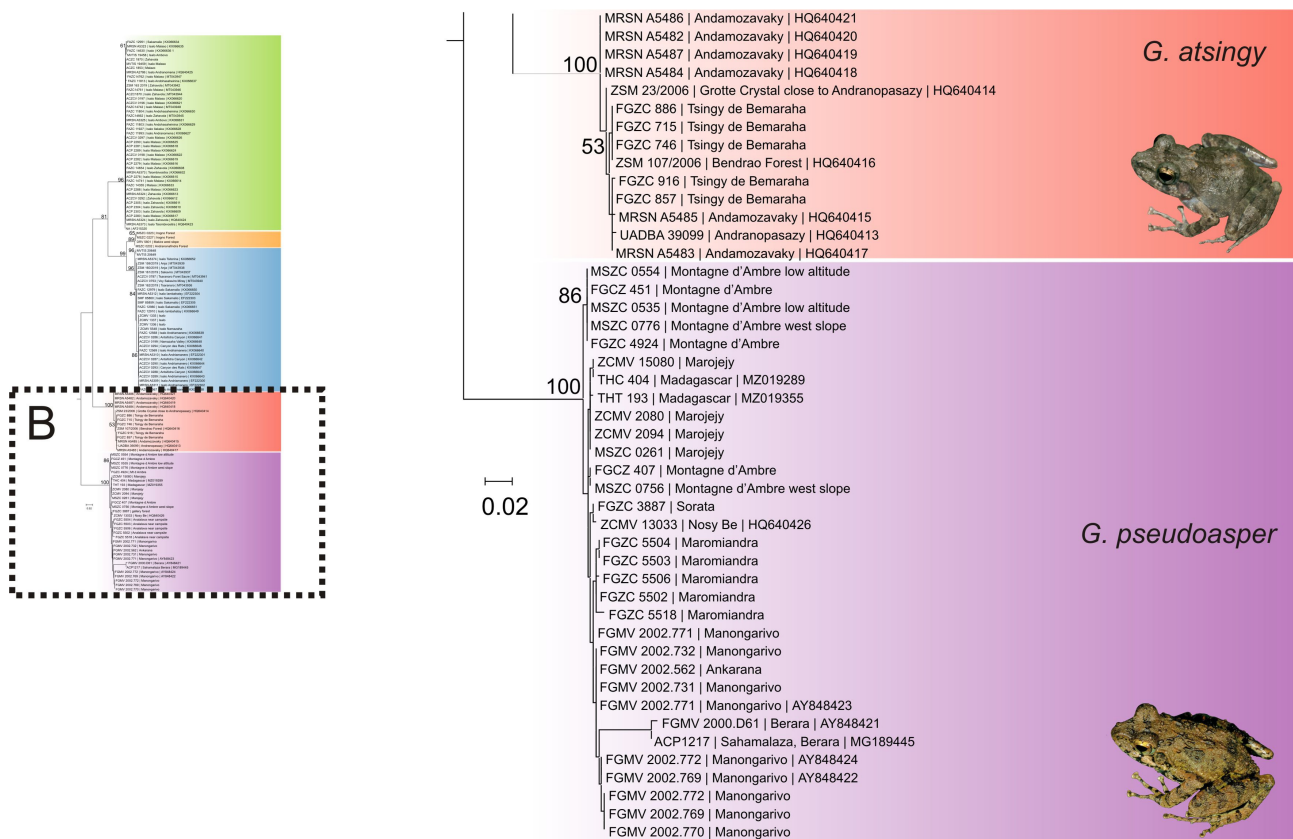
FIGURE 1. (Continued)

Taxonomy

Translating the new data available of the *G. corvus*-like frogs from the Sofia Region into a classification is a convoluted task. There seems to be no doubt that these frogs form the sister taxon to *G. corvus*, as suggested by mitochondrial and nuclear gene genealogies, and haplotype sharing in two out of four nuclear-encoded genes studied. However, given the absence of obvious morphological differences, and current unavailability of bioacoustic data which could provide indications of reproductive isolation, we currently lack evidence for a convincing species-level delimitation from *G. corvus.* Given the relatively high genetic divergence of 4.8–5.3% in 16S (which however is distinctly lower than among established *Phylacomantis* species), lack of haplotype sharing in two out of four nuclear-encoded genes studied, and apparently discontinuous distribution at about 850 km from the range of *G. corvus* in Isalo and about 750 km from the other reliably known sites (Tsaranoro, Anja, and Sakaviro), it is very well possible that these populations from the Sofia Region are already diverged at the species level. However, as has been previously argued (de Queiroz 2020; Scherz *et al.* 2022), in some cases the lack of complete congruence among lines of evidence may instead suggest incipient or incomplete speciation. The distinction between species and subspecies following this philosophy on one hand, and deep conspecific lineages and unconfirmed candidate species of Vieites *et al.* (2009) on the other hand, is a subtle one. The key here is that there is some, but not overwhelming, evidence from both mitochondrial and nuclear genetic data indicating divergence, and an absence of evidence for admixture or discordance; the biogeographic gap is enormous, and we know of no sites where the two lineages might co-occur, meaning that co-occurrence of both deeply divergent lineages in sympatry is, in our opinion, unlikely. As argued by Dufresnes *et al.* (2023) and Vences *et al.* (2024), naming the deepest intraspecific lineages with Linnaean scientific names rather than informally naming them as deep conspecific lineages, Evolutionary Significant Units, or Operational Taxonomic Units, makes sense to make them accessible for conservation legislation and management. And cautiously naming them as subspecies might be preferable to avoid taxonomic inflation and yet acknowledge and thus be better able to track, manage, and study this diversity. Also, this will allow their elevation to full species rank once more conclusive evidence becomes available.
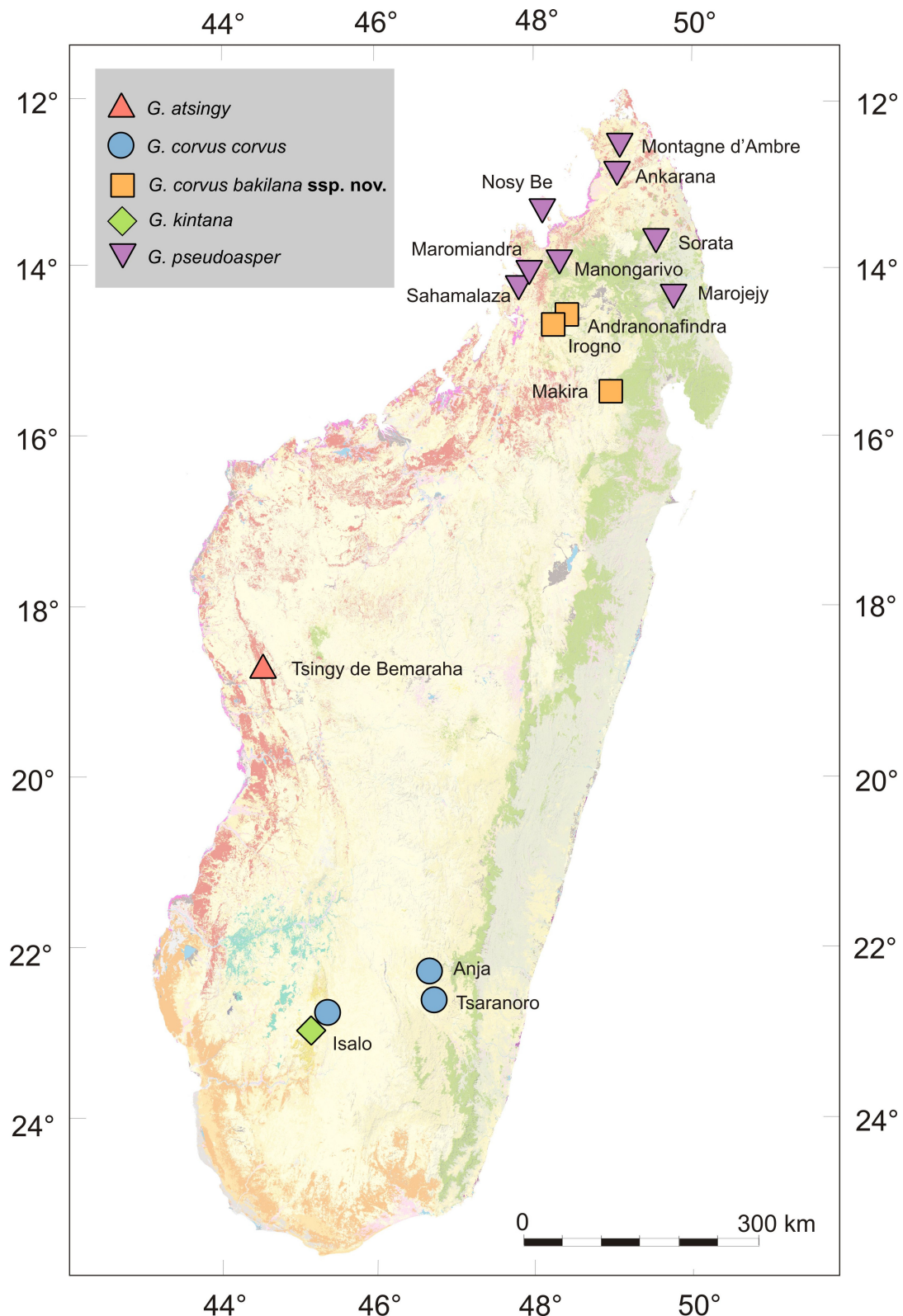
FIGURE 2. Map of Madagascar showing collection sites of genotyped samples of *Gephyromantis* (*Phylacomantis*) used in this study. Note that the sites represent the entire known ranges of the respective species except for *G. pseudoasper*, which has been collected also in other sites of northern and northeastern Madagascar, for *G. atsingy*, which has also been recorded from Beanka, and for *G. corvus*, which has been recorded from the Makay massif (record in need of confirmation). Basemap shows vegetation types according to the CEPF Madagascar Vegetation Mapping Project (https://web.archive.org/web/20170615094352/http://vegmad.org/). Colors represent the following vegetation types: light green = humid forests, red = dry deciduous forests, orange = spiny forest, light blue = western subhumid forests; light yellow = grassland.
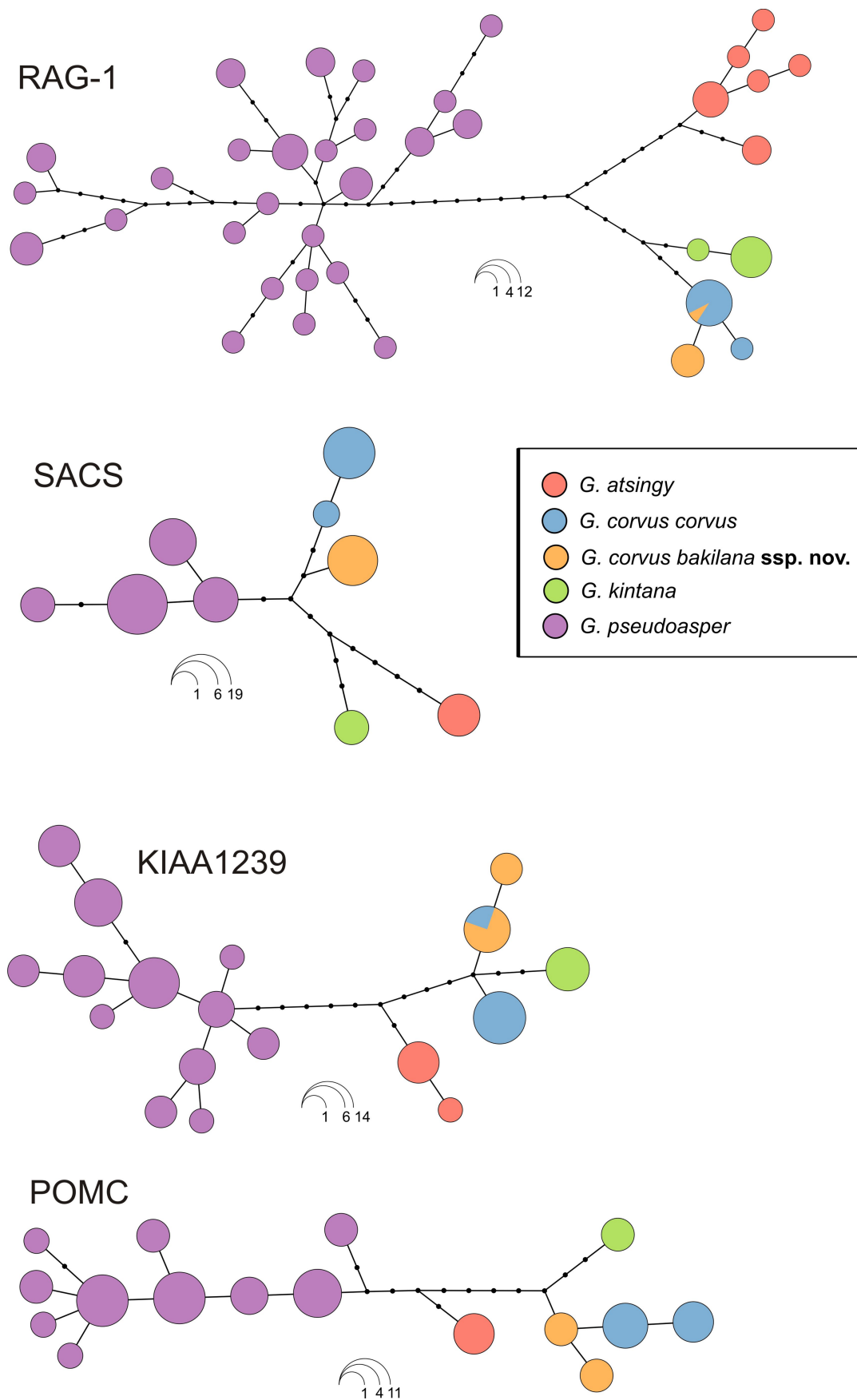
FIGURE 3. Haplotype networks of phased sequences of four nuclear-encoded gene fragments in species of *Gephyromantis* (*Phylacomantis*): RAG-1 (817 nucleotides, 36 samples), POMC (380 nt, 31 samples), SACS (568 nt, 28 samples), KIAA1239 (827 nt, 41 samples). Small black dots represent additional mutational steps or unsampled alleles.
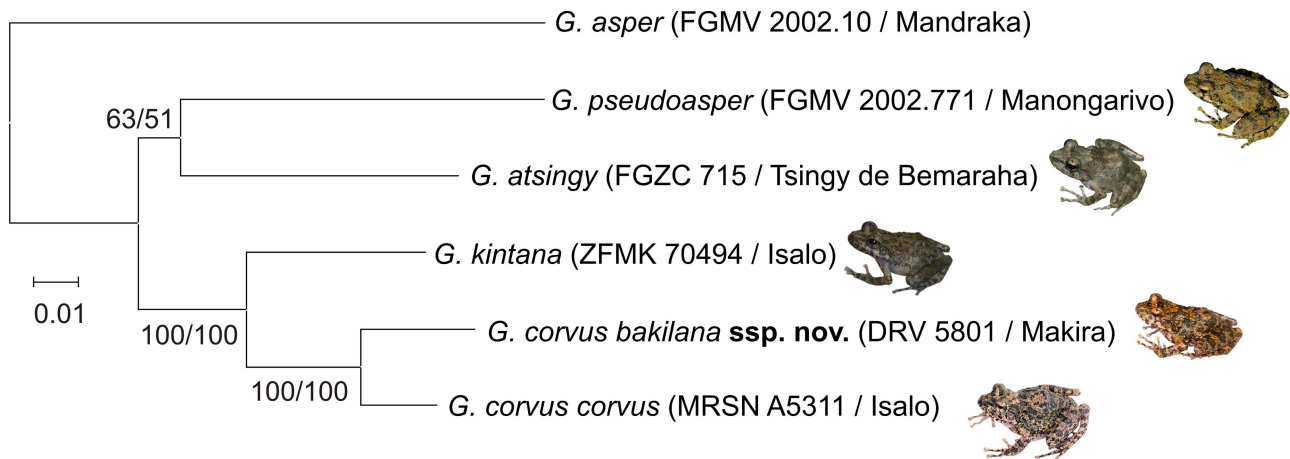
FIGURE 4. Maximum likelihood phylogenetic tree showing relationships among species of *Phylacomantis* based on concatenated sequences (6862 nucleotides) of four mitochondrial (12S and 16S rRNA, COB, COX1) and four nuclear-encoded gene fragments (RAG-1, POMC, SACS, KIAA1239). The tree was rooted with sequences of *Gephyromantis asper* (subgenus *Asperomantis*). Numbers at nodes are bootstrap proportions in percent (500 replicates) from an unpartitioned analysis (with RAxML, GTR+G model, also used for the tree itself) and a partitioned analysis (with IQ-TREE).

Hence, we here name and describe the newly discovered *Gephyromantis* (*Phylacomantis*) populations from the Sofia Region of Madagascar as a new subspecies of *G. corvus*, and emphasize the importance of additional fieldwork in this part of the island to obtain bioacoustic and natural history data on these frogs.

Gephyromantis corvus bakilana ssp. nov.
Figs. 5–6

Remark. This lineage was assigned the candidate species number *G*. sp. Ca31 by Scherz *et al.* (2017).

Holotype. ZSM 63/2016 (field number MSZC 202), adult male (Figs. 5–6), collected on 14 January 2016 by M.D. Scherz and M. Rakotondratsima at Andranonafindra forest (30 km SW of Bealanana on the RN31), geographical coordinates 14.73654°S, 48.54845°E, 1204 m a.s.l., Sofia Region, northwestern Madagascar.

Paratypes. ZSM 64/2016 (MSZC 227) and UADBA-MSZC 223, two adult females, collected on 16 January 2016 by M.D. Scherz and M. Rakotondratsima at Irogno forest, on the border of the Bealanana District (36 km SW of Bealanana on the RN31), geographical coordinates 14.74999°S, 48.49195–48.49209°E, 933–947 m a.s.l., Sofia Region, northwestern Madagascar; ZSM 505/2009 (DRV 5801), an unsexed adult, collected on 21 June 2009 by M. Vences, D. R. Vieites, F. M. Ratsoavina, R.-D. Randrianiaina, E. Rajeriarison, T. Rajoafiarison, and J. Patton at Sahaovy (camp 0), outside but close to the western border of the Makira Natural Park, geographical coordinates 15.48904°S, 49.07854°E, 603 m a.s.l., Sofia Region, northeastern Madagascar.

Diagnosis. A subspecies of *Gephyromantis* assigned to the subgenus *Phylacomantis* based on the combination of presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (assessed by external examination), absence of nuptial pads, a single patch of well-delimited femoral glands of type 2 (*sensu* Glaw *et al.* 2000) in males (no rudiments in females), paired or bilobed blackish subgular vocal sacs in males, tubercular skin without regular dorsolateral ridges, medium body size, outer metatarsalia partly connected by tissue, webbing between toes present, inner and outer metatarsal tubercle present, and molecular phylogenetic affinities.

Within the subgenus *Phylacomantis*, *G. c. bakilana* **ssp. nov.** is distinguished from *G. pseudoasper* by less expressed yellow-orange coloration ventrally on thighs and on femoral glands (vs. usually distinct yellow-orange color), and less distinct median white stripe on throat (vs. usually distinct stripe); from *G. kintana* by blackish (vs. whitish) vocal sacs, by blackish (vs. whitish) throat, by a blackish (vs. whitish) belly, more strongly granular dorsal skin (vs. less granular), and lower maximum number of granules in the femoral glands (ca. 45 vs. 96); and from *G. atsingy* by brownish dorsal coloration with orange-brown elements (vs. light brown-beige with a greenish

shading) and a lower maximum number of granules in the femoral glands (ca. 45 vs. 70). Morphologically, the new subspecies does not show any known consistent differences to *G. corvus corvus*; one potential difference is less-expressed dorsal orange-brown color pattern in *G. c. corvus*, but the holotype was fairly dark when photographed, and more samples are needed to confirm this. According to MolD, the following combination of nucleotide position is diagnostic for the new subspecies among all other taxa in the subgenus *Phylacomantis*, including nominal *G. c. corvus*: (positions according to the full 16S sequence of *G. pseudoasper*, AB325880): "A" at site 1019, "G" at site 1054.

Description of the holotype. Adult male in good state of preservation (Fig. 6), with part of the ventral surface of left thigh cut and opened for tissue sampling and femoral gland inspection; skin on right thigh also cut. SVL 39.1 mm; for other measurements see Table 2. Body somewhat stout; head longer than wide, slightly narrower than body; snout rounded in dorsal and lateral views; nostrils directed laterally, much nearer to tip of snout than to eye; canthus rostralis well defined, straight; loreal region concave; tympanum distinct, rounded, its horizontal diameter 60% of eye diameter; supratympanic fold distinct, regularly curved from eye to posterior edge of tympanum, then running straight towards forelimb insertion; tongue ovoid, distinctly bifid posteriorly; vomerine teeth present as two distinct rounded aggregations posteromedially from choanae; choanae rounded; maxillary teeth present. Vocal sacs on throat clearly recognizable as distinct lateral skin folds, the right sac partly distended, the left sac not distended. Arms slender; subarticular tubercles single; paired outer and unpaired inner metacarpal tubercles present and distinct; fingers without webbing; finger disks triangular, distinctly enlarged; nuptial pads absent. Hind limbs slender; tibiotarsal articulation reaching between nostrils and snout tip when hindlimbs are adpressed along body; lateral metatarsalia partly connected; inner metatarsal tubercle distinct, outer metatarsal tubercle small but distinct; webbing of foot 1(1), 2i(1.5), 2e(1), 3i(2), 3e(1.25), 4i(2.5), 4e(2.25), 5(1). Skin distinctly granular on dorsum, with a series of irregular longitudinal rows of tubercles and discontinuous ridges. Ventrally, smooth on throat and chest and finely granular on belly. Femoral macrogland ("Type 2", according to Glaw *et al.* 2000) distinctly recognizable in internal and external view, containing ca. 45 single gland granules.

After 7 years in ethanol (Fig. 6), dorsum almost uniformly brown, washed with some poorly contrasted darker and lighter tones. Upper and (more distinctly) lower lip with alternating light and dark bars (the light bars distinctly narrower). Hindlimbs dorsally brown with 4–5 dark brown crossbands on shank and thigh. Flanks dark brown with whitish spotting. Belly cream with irregular brown mottling. Vocal sacs blackish-gray. In life (Fig. 5), color was similar, with a predominance of dark brown color dorsally and ventrally. Eyes had a grayish brown iris, with a vertical dark brown bar medially both in its upper and lower half.

Variation. The two female paratypes for which measurements are available agree morphologically with the male holotype (Table 2), but are slightly smaller (SVL 38.0–38.5 vs. 39.1 mm); they lack femoral glands and vocal sacs. In life, all three paratypes (Fig. 5) had orange-brown color elements dorsally. ZSM 505/2009 from Makira had yellow color ventrally, especially in the inguinal region and on thighs and shanks (Fig. 5).

Etymology. The subspecies epithet is derived from the Malagasy noun "bakilana" = a "piece, scrap, morsel, or fragment", referring to the tiny fragments of forest where populations were discovered, and also referring to the identity of a subspecies as a putative fragment of a species-level lineage. The name is used as a noun in apposition.

Distribution. So far known only from three localities comprised by the type series (Andranonafindra Forest, Irogno Forest, Makira west slope). None of the known localities of *G. c. bakilana* **ssp. nov.** (Fig. 2) is situated in a protected area, although paratype ZSM 505/2009 was found only 5–6 km west and south of the borders of the Makira Natural Park and therefore might be present in the reserve. The known elevational range is 600–1200 m a.s.l.

Natural history. At Makira, specimens were observed along a rather large rocky stream in a largely deforested area, at the western edge of the reserve, while at other nearby sites within primary rainforest, no further specimens were found. At Andranonafindra, specimens were encountered in the early evening around 19–20 h along a narrow stream in an isolated valley forest fragment. One specimen (probably the holotype) was heard calling but could not be recorded. The forest fragment contained actively burning charcoal pits, and lies directly beside a large road built in order access the nearby hills for mining purposes. The hills nearby are largely denuded of forest except a narrow riparian strip, which however harbors a moderately diverse amphibian fauna. The Irogno specimen was found on a dead tree near rocks along a river.
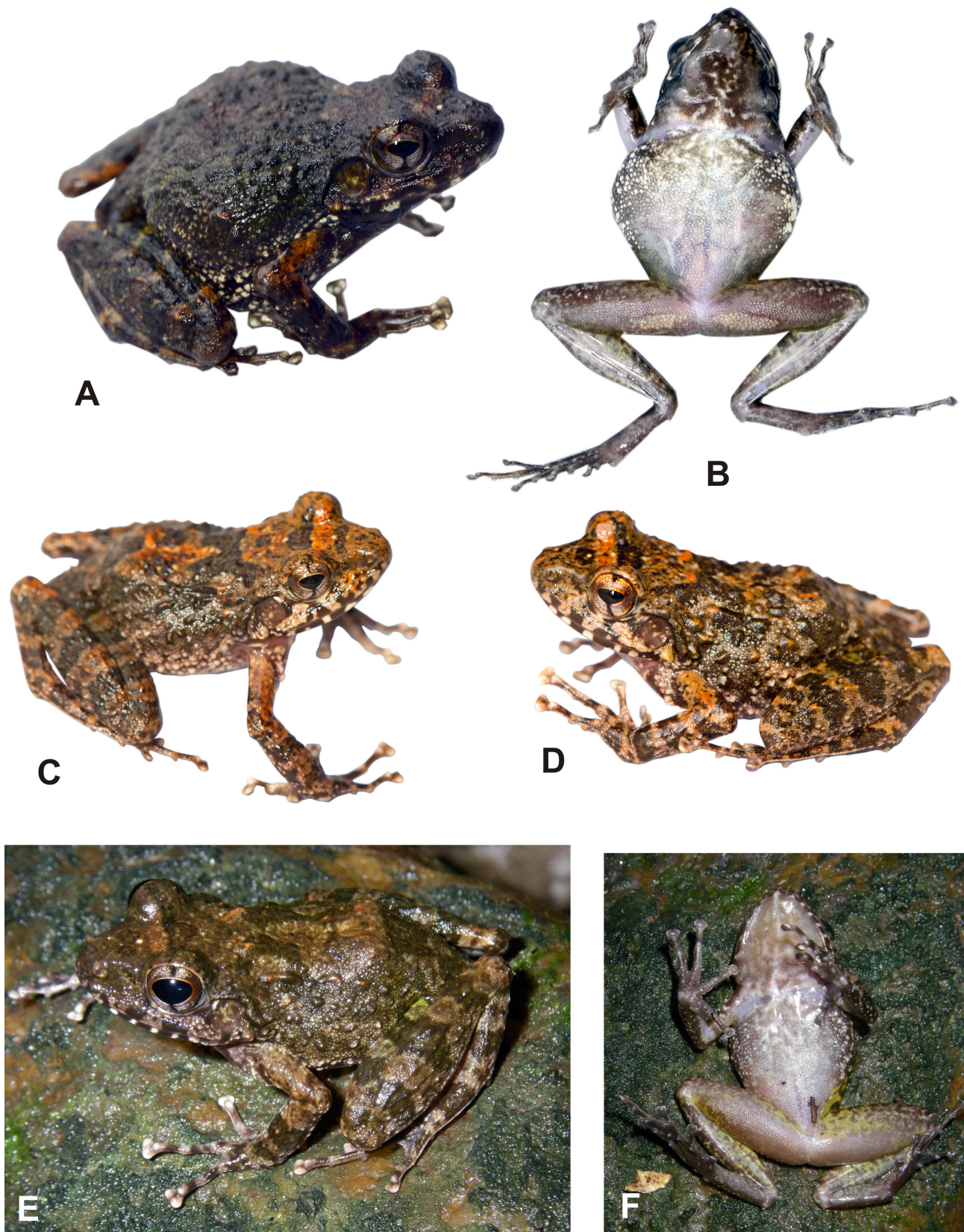
FIGURE 5. Photographs of type specimens of *Gephyromantis* (*Phylacomantis*) *corvus bakilana* **ssp. nov.** in dorsolateral and ventral views in life. A, B, male holotype ZSM 63/2016 (MSZC 202) from Andranonafindra forest. C, female paratype UADBA-MSZC 223 from Irogno forest; D, female paratype ZSM 64/2016 (MSZC 227) from Irogno forest; E, F paratype ZSM 505/2009 (DRV 5801) from near the Makira Reserve (western slope). Photographs not to scale.

FIGURE 6. Preserved holotype of *Gephyromantis* (*Phylacomantis*) *corvus bakilana* **ssp. nov.** (ZSM 63/2016; MSZC 202) in dorsal and ventral views.

TABLE 2. Morphometric measurements of holotype and two paratypes of *Gephyromantis corvus bakilana* **ssp. nov.** M, male; F, female; HT, holotype; PT, paratype. See Materials and Methods for other abbreviations.

Catalogue number	ZSM 63/2016	ZSM 64/2016	ZSM 505/2009
Field number	MSZC 202	MSZC 227	DRV 5801
Status	HT	PT	PT
Sex	M	F	F
SVL	39.1	38.5	38.0
HW	14.7	14.6	14.6
HL	16.5	16.6	15.5
TD	3.4	3.5	3.4
ED	5.8	5.2	5.3
END	3.3	2.9	3.7
NSD	2.4	2.4	2.5
NND	3.5	3.4	3.7
HAL	11.6	12.8	12.0
FORL	26.6	26.4	25.9
HIL	64.5	66.0	64.3
FOTL	28.3	30.5	28.0
FOL	18.9	20.9	18.7
TIBL	21.0	21.0	20.6
FGL	8.7	NA	NA
FGW	3.0	NA	NA

Discussion

This study provides a comprehensive overview of the molecular diversity of the subgenus *Phylacomantis*. Compared to the previous assessment of Cocca *et al.* (2020), which focused on *G. corvus* and *G. kintana*, the two species co-occurring in the Isalo region in the South, we here expanded the sampling for *G. pseudoasper* including samples from the North East and North regions (Marojejy, Sorata, Ankarana, and Montagne d'Ambre), thus demonstrating that this species only has a low amount of mitochondrial variation across its range and that it might have colonized the rainforest areas only relatively recently. We also provided the first population-level data of nuclear-encoded genetic variation in the subgenus *Phylacomantis*, demonstrating that all nominal species are clearly separated in all markers studied, without instances of haplotype sharing among them. This includes the syntopic *G. corvus* and *G. kintana*, thus providing additional conclusive evidence for their status as distinct species and for the lack of gene flow among them.

The distribution of the new subspecies *G. c. bakilana* is enigmatic given the apparent range gap of about 850 km to Isalo and the other genetically confirmed sites (Tsaranoro, Anja, and Sakaviro), where *G. c. corvus* occurs. Even if the species' occurrence in Makay (Rakotondravony & Goodman 2011) were to be confirmed by molecular data, the geographic gap would remain substantial. In general, the western slope of Makira and its vicinities are known to host species otherwise occurring in the West and South of Madagascar, such as *Boophis tampoka*, *Blommersia bara*, *Mantidactylus mahery*, and *M. ulcerosus* (Vences *et al.* 2011, 2023; Scherz *et al.* 2022). Of these, *B. bara* and *M. mahery* have ranges extending towards Isalo but also have been recorded from other sites in-between. Frog species such as *Laliostoma labrosum*, *Blommersia bara*, or *Boophis doulioti* have wide and continuous ranges from the South to the North West and Sambirano regions, and beyond in the case of *Laliostoma*, as far as known without a pattern of strong genetic structure concordant between mitochondrial and nuclear genes (Vences & Glaw 2002; Pabijan *et al.* 2015; Rodríguez *et al.* 2015; Vences *et al.* 2023), and other taxa such as the *Scaphiophryne calcarata* complex are also continuously distributed but consist of unambiguous and clearly different species (Scherz *et al.* 2021). The case of *G. corvus* is exceptional as it so far is known - with two genetically distinct subspecies - only from sites in the North East and North West biogeographic regions (*G. c. bakilana*) and the South (*G. c. corvus*), without any population known from the area in between. A similar case is the species pair *Boophis occidentalis* (occurring in the South including the borders to the eastern rainforests, Isalo and Makay but also including Bemaraha) and *Boophis tsilomaro* (apparently restricted to Sahamalaza in the NW) (Vences *et al.* 2010). The rarity of this pattern may also be a reflection of the poor herpetological exploration of areas in the ecotone separating the humid eastern and more arid western biomes in Madagascar's central highlands, a largely deforested area with only very little remaining primary vegetation. Additional fieldwork in these sites, which are logistically challenging to survey, may yield additional and perhaps equally surprising biogeographical insights as the case of *G. corvus*.

Under the current assessment (IUCN SSC Amphibian Specialist Group 2016), *G. corvus* is considered as Endangered under criteria B1ab(iii). This is based on (i) its range (extent of occurrence) estimated to be less than 5,000 km^2 and severely fragmented or no more than five locations are known, and (ii) continuing decline, observed, inferred or projected, in area, extent and/or quality of habitat (i.e., within Isalo National Park and the other forest fragments in which it was known to occur, such as Tsaranoro, Anja, and Sakaviro). Here, we propose to consider the newly discovered *Gephyromantis* (*Phylacomantis*) populations from the Sofia Region of northern Madagascar as a subspecies of *G. corvus*, which increases the species' overall extent of occurrence from 2589 km^2 (IUCN SSC Amphibian Specialist Group 2016) to >70,000 km^2. However, the known forest fragments in the Sofia Region are small, suggesting a small area of occupancy for the species, all of which is under strong risk of deforestation, while the species' range continues to be severely fragmented. We therefore consider it to remain at a high level of threat, and recommend it be re-assessed as soon as possible. Furthermore, we recommend that, once *G. corvus* is re-assessed, both subspecies should receive their own assessments, following IUCN Red List Guidelines, helping to ensure that *G. c. bakilana* can be considered for conservation management, and to ensure an assessment is already present if it is later elevated to species level.

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