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Integrative revision of the *Blommersia wittei* complex, with description of a new species of frog from western and north-western Madagascar

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Abstract

Frogs of the *Blommersia wittei* complex are widespread in western and northern Madagascar, and are one of two clades of the family Mantellidae that have colonized the Comoran island of Mayotte. Based on a comprehensive set of DNA sequences of the mitochondrial 16S rRNA gene and the nuclear-encoded RAG1 and SACS genes, integrated with morphological and bioacoustic data, we here analyze the genetic differentiation of populations of this complex across Madagascar. We confirm that a candidate species named *B.* sp. Ca5 in previous studies represents a genetically well-defined evolutionary lineage distributed over much of western Madagascar, which we describe herein as *Blommeria bara* **sp. nov.** based on its molecular and bioacoustic differentiation. *Blommersia wittei* occurs across northern Madagascar but its type locality Ambanja, at the lower Sambirano river, is very close to the range of another, newly discovered microendemic lineage that was only found at two sites along the upper Sambirano river (here named as candidate species *B.* sp. Ca12). The *B. wittei* complex thus provides an example of a clade of closely related Malagasy frogs that contains species widespread over hundreds of kilometers, as well as extreme microendemics. For a full resolution of this species complex, more data need to be collected on the geographical contact among these two lineages, on the morphology and bioacoustics of *B.* sp. Ca12, and on the north-eastern populations of *B. wittei* at Sambava, which are weakly differentiated in mitochondrial genes but differ in bioacoustics and possibly in the extent of foot webbing.

Key words: Amphibia, Anura, Mantellidae, *Blommersia bara* **sp. nov**., microendemism, species delimitation

Introduction

Madagascar harbors a rich fauna of anuran amphibians, which in 2023 has exceeded 400 recognized and named species (AmphibiaWeb 2023). The majority of these species are found in the rainforests of the eastern escarpment and of the northern Sambirano region. This is in line with most other Madagascar-endemic vertebrate radiations

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whose species richness is strongly influenced by the colonization—or not—of these rainforests (Crottini *et al.* 2012). A number of species also occur in the more arid West and South-West of the island (Blommers-Schlösser 1979; Blommers-Schlösser & Blanc 1993; Glaw & Vences 2007), the majority of which live in small relict humid forests such as in the canyons of the Isalo limestone massif (Mercurio *et al.* 2008; Cocca *et al.* 2018) or the Tsingy de Bemaraha limestone karst (Bora *et al.* 2010). However, several species have adapted specifically to arid environments, such as *Scaphiophryne brevis*, *S. calcarata*, *S. obscura*, *Dyscophus insularis*, or *Laliostoma labrosum*, all of which are rather robust species that evade desiccation during dry episodes by burrowing into the ground (Blommers-Schlösser & Blanc 1991; Glaw & Vences 2007; Pabijan *et al.* 2015; Scherz *et al.* 2021). Also, some partly arboreal or scansorial species such as *Heterixalus luteostriatus* and *H. tricolor*, *Boophis doulioti* and *B. xerophilus*, and a representative of the genus *Blommersia*, historically referred to as *B. wittei* (e.g., populations from Ankarafantsika and Namoroka in Blommers-Schlösser & Blanc 1991), are widespread in relatively arid areas of western Madagascar. More recently the latter frogs were assigned to a confirmed candidate species, *B.* sp. Ca5, due to their strong genetic differentiation and apparent bioacoustic differentiation compared to *B. wittei* specimens from areas near the species' type locality Ambanja in the northern Sambirano region (Vieites *et al.* 2009).

Taking into account the latest species description (Vences *et al.* 2023), the genus *Blommersia* currently contains 11 species from Madagascar, and two species from the Comoran island of Mayotte (AmphibiaWeb 2023). *Blommersia* belongs to the family Mantellidae, an ecomorphologically diverse group endemic to Madagascar and the Comoros (Glaw & Vences 2006), and consists of small to medium-sized semi-arboreal species that breed in lentic, often temporary water bodies. *Blommersia* are morphologically relatively inconspicuous frogs, and the first three species of the genus—including *B. wittei*—were described only in 1974 (Guibé 1974). *Blommersia wittei* is a relatively common species at low elevations in northern Madagascar, where its calls are often heard in the rainy season from temporary swamps. Molecular data place the species into a clade with *B.* sp. Ca5 from western Madagascar, and with the Comoran species, *B. nataliae* and *B. transmarina* (Vieites *et al.* 2009, 2020; Wollenberg *et al.* 2011; Vences *et al.* 2023).

In this study, we analyze the evolutionary relationships and taxonomy of frogs assigned to *B. wittei* and *B.* sp. Ca5 based on combined evidence from DNA sequences, advertisements calls and morphology from a comprehensive sampling across the ranges of these frogs.

Material and Methods

Voucher specimens and tissue samples were collected during field expeditions in Madagascar between 2000–2018. Frogs were caught during nocturnal and diurnal searches, either opportunistically by catching animals on the ground around swamps, or at night by locating calling males. Specimens were anesthetized by immersion in MS222 or chlorobutanol solution, and subsequently euthanized with an overdose of the same substances. Tissue samples for molecular analysis, typically parts of thigh muscles, were taken and stored in 1.5 ml vials with pure ethanol. Voucher specimens were fixed in 95% ethanol and preserved in 70% ethanol, and deposited in the following collections: Museo Regionale di Scienze Naturali, Torino, Italy (MRSN), Université d'Antananarivo, Département de Biologie Animale (UADBA), Zoological Museum Amsterdam (ZMA; collections now in Naturalis, Leiden), and Zoologische Staatssammlung München (ZSM). Additionally, type material from the Muséum national d'histoire naturelle, Paris (MNHN) was studied. We use ACZC, ACP, DRV, FA, FAZC, FGZC, FGMV, ZCMV, MSZC, and THC to refer to field numbers of A. Crottini, D. Vieites, F. Andreone, F. Glaw and M. Vences, M. D. Scherz, and T. R. Fulgence, 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 DOI 10.5281/zenodo.8049142. Note that most vouchers deposited in UADBA have not been catalogued in that collection and therefore have no final catalogue numbers yet. In addition several sequences are from unvouchered tissue samples, held in the collection of A. Crottini (ACP series).

TABLE 1. Morphometric measurements (all in mm) and femoral gland conditions of adults of the *Blommersia wittei* species complex targeted in this study. For abbreviations of measurements, see Materials and Methods; other abbreviations: HT, holotype; PT, paratype; F, female; M, male; FG, femoral gland. NA, not applicable; NM, not measured.

Catalogue number	Field number	Locality	Status	Sex	SVL	HW	HL	HTD	HED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIBL	FGL	FGW
B. wittei																				
ZSM 51/2018	MSZC 404	Montagne d'Ambre	PT	F?	25.4	8.6	9.8	2.2	3.2	3.3	1.3	2.7	16.0	7.4	39.4	18.8	12.9	13.0	NA	NA
ZSM 50/2018	MSZC 521	Montagne d'Ambre	PT	M	22.6	7.3	9.2	2.1	2.8	2.5	1.6	2.4	13.9	6.8	35.1	16.3	10.6	11.1	4.9	2.1
ZSM 384/2016	ZCMV 15039	Sambava	PT	F?	21.3	6.4	8.5	1.8	3.0	2.3	1.5	2.4	13.4	6.7	35.6	16.4	10.9	11.0	NA	NA
ZSM 563/2000	FGMV 2000.378	Sambava	PT	M	24.7	7.5	9.1	2.4	2.8	2.3	1.5	2.4	14.6	7.3	40.3	19.1	13.1	12.4	4.3	2.3
ZSM 411/2000	FGMV 2000.121	Ambanja	PT	M	24.3	7.9	9.7	2.0	3.3	2.3	1.8	2.8	14.7	7.3	39.6	18.1	12.0	11.6	3.5	2.0
ZSM 405/2000	FGMV 2000.443	Benavony	PT	F	24.6	7.4	9.4	2.0	3.0	2.4	1.4	2.8	14.4	6.7	39.3	18.2	12.7	12.0	NA	NA
ZSM 2229/2007	FGZC 1387	Forêt d'Ambre	PT	M	23.4	7.3	9.0	2.0	2.9	2.3	1.2	2.6	14.9	7.4	36.8	17.0	11.7	11.7	4.6	2.0
B. bara **sp. nov.** (*B.* sp. Ca5)			PT																	
ZSM 31/2004	FGZC 57	Isalo	HT	M	18.5	5.8	7.1	1.7	2.7	2.2	1.4	2.3	11.5	5.3	28.6	14.0	9.4	9.0	3.8	1.9
ZSM 2284/2007	ZCMV 5801	Isalo	PT	M	20.5	6.3	7.7	2.0	2.8	2.1	1.4	2.0	12.6	6.2	NM	15.0	10.2	9.8	4.4	1.9
ZSM 22/2004	FGZC 34	Isalo	PT	M	18.2	5.6	7.5	1.8	2.3	2.0	1.1	2.0	12.1	5.4	30.4	13.9	9.4	9.0	3.8	1.9
ZSM 24/2004	FGZC 38	Isalo	PT	M	18.4	6.3	7.1	1.8	2.4	2.2	1.7	2.2	11.4	5.3	30.3	13.3	8.7	9.0	4.5	2.2
ZSM 2285/2007	ZCMV 5804	Isalo	PT	F	22.0	6.3	8.1	2.0	2.7	2.4	2.0	2.4	13.2	6.5	34.0	16.1	10.5	10.4	NA	NA
ZSM 3222/2012	ZCMV 14143	Mariarano	PT	M	22.8	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
ZSM 247/2003	NA	Kirindy	PT	M	23.3	7.0	9.0	1.7	3.4	2.0	1.9	2.4	14.4	7.0	34.4	16.6	11.6	10.7	4.9	2.6
ZSM 246/2003	NA	Kirindy	PT	M	23.3	7.3	8.8	2.0	3.2	2.6	1.8	2.8	15.6	7.5	38.0	17.7	11.6	11.9	5.0	2.6
ZSM 52/2006	FGZC 779	Tsingy de Bemaraha	PT	M	23.3	7.6	8.8	2.1	3.3	2.2	1.6	2.7	14.9	7.4	38.3	17.3	11.7	11.3	4.8	2.5
ZSM 30/2006	FGZC 727	Tsingy de Bemaraha	PT	M	24.0	7.4	9.4	2.0	3.0	2.3	1.7	2.8	15.0	7.4	37.4	17.7	11.6	11.5	4.9	2.0
ZSM 13/2006	FGZC 691	Tsingy de Bemaraha	PT	F	26.4	7.7	9.7	2.2	3.3	2.5	1.8	2.5	16.3	7.8	40.3	19.2	13.4	12.6	NA	NA
ZSM 706/2001	FGMV 2001.279	Ankarafantsika	PT	M	23.4	7.3	9.1	2.1	2.9	2.6	1.6	2.4	14.4	7.6	37.7	18.2	12.7	11.3	4.0	2.0
ZSM 2281/2007	ZCMV 5625	Ankarafantsika	PT	M	25.7	7.7	9.6	2.4	3.2	2.3	1.5	2.4	16.2	8.2	42.1	19.5	13.4	12.9	NA	NA
ZSM 2320/2007	ZCMV 5621	Ankarafantsika	PT	M	25.6	7.4	9.7	1.9	3.3	2.4	1.5	2.5	15.9	7.6	39.7	18.4	12.7	12.4	5.3	2.6
ZSM 2283/2007	ZCMV 5643	Ankarafantsika	PT	F	24.8	7.9	9.2	2.0	3.1	2.5	1.6	2.4	15.7	8.1	41.6	18.4	12.6	12.9	NA	NA
B. sp. Ca12			PT																	
ZSM 589/2001	FGMV 2001.34	Antsirasira	PT	M	23.2	7.0	8.9	2.0	3.0	2.8	1.7	2.4	14.7	6.9	38.9	18.1	12.2	12.0	3.4	2.0

The following measurements were taken by MV using a manual caliper at an accuracy of 0.1 millimeter: snout-vent length (SVL); maximum head width (HW); head length from posterior edge of snout 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 base of the hand to the tip of the longest finger (HAL); hind limb length, from the cloaca to the tip of the longest toe (HIL); foot length (FOL); foot length including tarsus (FOTL); tibia length (TIBL); femoral gland length (FGL); and femoral gland width (FGW). Geographical regions within Madagascar are named according to Boumans *et al.* (2007) and Brown *et al.* (2016).

We recorded anuran vocalizations in the field using different digital or analogue devices such as Sony WM-D6C and Tensai RCR-3222 tape recorders with external microphones (Sennheiser Me-80, Vivanco EM 238), and Tascam DR07, DR05, Marantz PMD 660 or Roland Edirol R-09 digital recorders, with built-in microphones (Tascam) or accessorized with semi-directional microphones (Marantz and Roland). We obtained digital recordings at a sampling rate of 44.1 kHz and 24-bit resolution and saved them as uncompressed files. Recordings were digitized or resampled at 22.05 kHz and 32-bit resolution and computer-analyzed using the software CoolEdit Pro 2.0. Frequency information was obtained through Fast Fourier Transformation (FFT; width 1024 points) with the Hanning window function; audiospectrograms were drawn with the Blackman window function with 256 bands resolution. Temporal characters were measured from oscillograms. Measured numerical call parameters were rounded and are provided as range followed by mean ± standard deviation in parentheses. Terminology of call descriptions and methods for call analyses follow those recommended by Köhler *et al.* (2017), using the call-centered terminological scheme. All recordings were high-pass filtered at 600 Hz to remove unwanted low-frequency background noise. In all cases, filtering was exclusively applied to frequencies outside the bandwidths of calls. For the purpose of easy and immediate comparability, audiospectrograms and corresponding oscillograms of advertisement calls of all populations are shown at a time scale of 600 ms.

DNA was extracted from ethanol-preserved tissue samples using a standard salt extraction protocol (Bruford *et al.* 1992). We DNA barcoded available and not previously sequenced tissue samples assigned to *B. wittei* and *B.* sp. Ca5 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), including *Blommersia* (e.g., Glaw & Vences 2002; Vences *et al.* 2010; Pabijan *et al.* 2011; Glaw *et al.* 2019; Vieites *et al.* 2020). The DNA fragment was PCR-amplified 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. We furthermore sequenced a subset of samples for a fragment of the nuclear-encoded recombination activating gene 1 (RAG1), 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 used the sequencing primer RAG1-Manti-Seq1 (5'-GCAAAGCCVTTTATTGAAACC-3'). Finally, a subset of samples was sequenced for a fragment of sacsin (SACS). Amplifications of the SACS fragment were performed using a nested approach (Shen *et al.* 2012) using external primers SACSF2 (5'-AAYATHACNAAYGCNTGYTAYAA-3') and SACSR2 (5'-GCRAARTGNCCRTTNACRTGRAA-3') and internal primers SACSNF2 (5'-TGYTAYAAYGAYTGYCCNTGGAT-3') and SACSNR2 (5'-CKGTGRGGYTTYTTRTARTTRTG-3') and with cycling protocol for both PCRs as follows: 94°C (240s), 45 x [94°C (45s), 45°C (40s), 72°C (120s)], 72°C (600s).

PCR products were purified with Exonuclease I and Shrimp Alkaline Phosphatase digestion and sent to LGC Genomics (Berlin) for sequencing on automated DNA sequencers. 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: OR123906–OR123961 and OR127037–OR127116).

Sequences were aligned using the Clustal algorithm implemented in MEGA7 (Kumar *et al.* 2016), and a phylogeny inferred under the Maximum Likelihood (ML) optimality criterion, after selecting the most appropriate substitution model based on the Bayesian Information Criterion. Node support was assessed with 500 bootstrap replicates.

For graphically representing the relationships among alleles (haplotypes) of the RAG1 and SACS gene

fragments, we used a network approach. Alleles (haplotypes) were inferred with the PHASE algorithm (Stephens *et al.* 2001) implemented in the DnaSP software (Version 5.10.3; Librado & Rozas 2009), an ML tree inferred under the Jukes-Cantor substitution model in MEGA7 (choosing this simple model to avoid over-parametrization), and this tree and the respective alignment were used as input for Haploviewer (written by G. B. Ewing; http://www.cibiv.at/~greg/haploviewer), a software that implements the methodological approach of Salzburger *et al.* (2011).

Pairwise genetic distances were calculated from the 16S alignment using TaxI2 (Vences *et al.* 2021). Species partitions were inferred using ASAP (Puillandre *et al.* 2021) and subsequently we compared the favored ASAP partition for concordance with evidence from RAG1 and SACS differentiation.

Results

Molecular differentiation and evolutionary relationships

The ML tree inferred from the alignment of the mitochondrial 16S fragment (alignment length 533 bp; 127 ingroup samples; T92+G model) revealed several deep lineages within the *B. wittei* complex (Fig. 1). A lineage containing samples assigned to *B. wittei*, including samples from the type locality Ambanja, was sister to a clade including the two Comoran species, *B. nataliae* and *B. transmarina*, which are not further discussed herein. Samples in the *B. wittei* lineage all originated from northern Madagascar (as defined by Brown *et al.* 2016). Two other mitochondrial lineages split from nodes basal to this (*wittei* (*nataliae*, *transmarina*)) clade, one originating from a small area in the Sambirano region, and the other from the West and South West of Madagascar (Fig. 2). We emphasize that this 16S tree is here mainly presented for the purpose of DNA barcoding, i.e., assigning samples to main mitochondrial lineages. In fact, the short 16S fragment is not sufficient to reliably resolve deeper phylogenetic relationships among the species involved (for a multi-gene tree with a hypothesis of the placement of the *B. wittei* complex within *Blommersia*, see Vences *et al.* 2023).

The analysis with ASAP confirmed the existence of various species-level lineages in our data set. The species partition with the best (lowest) ASAP score of 1.0 supported five ingroup partitions, corresponding to (1) *B. nataliae*, (2) *B. transmarina*, (3) *B. wittei* (including the somewhat deviant samples from Sambava that will be discussed below), (4) the microendemic Sambirano lineage for which we here coin the candidate species name *Blommersia* sp. Ca12, and (5) the lineage from the West and South West, which corresponds to the previously named candidate species *B.* sp. Ca5. The second-best species partition (ASAP score = 2.0) merged *B. transmarina* with *B. wittei*, which is an unplausible result given the clear differences between these two species, and the third best partition (ASAP score = 4.5) separated samples from Sambava from other *B. wittei* samples into an additional sixth ingroup subset.

Considering the partition with five lineages, genetic distances between the main lineages were high. Uncorrected pairwise distances in the 16S fragment were 5.0–8.2% between *B. wittei* and *B.* sp. Ca5, 5.5–7.0% between *B. wittei* and *B.* sp. Ca12, and 4.9–6.4% between *B.* sp. Ca5 and Ca12. The Sambava specimens differed from other *B. wittei* by a small divergence of 1.2%. The two Comoran species differed from the Malagasy lineages by a minimum of 4.6% (between *B. transmarina* and *B. wittei*), and by 5.1% from each other (a slightly inflated value because the *B. nataliae* sequence is rather short, thus with overrepresentation of highly variable stretches).

The haplotype networks reconstructed from phased alleles of fragments of the nuclear-encoded genes for RAG1 (33 samples, 457 bp) and SACS (23 samples, 924 bp) revealed that each of the Malagasy mitochondrial lineages (Comoran taxa were not included) formed independent phylogroups also from the nuclear perspective, in general without haplotype sharing among lineages (Fig. 1), with the exception, in the RAG1 network, of the shared alleles between *B. wittei* and *B.* sp. Ca12. Additionally, also in the RAG1 network, some *B. wittei* samples showed rather divergent alleles that may represent sequencing errors or sequence contamination (two of the four sequences in this deviant phylogroup were from individuals whose second allele was placed in the regular *wittei* phylogroup).

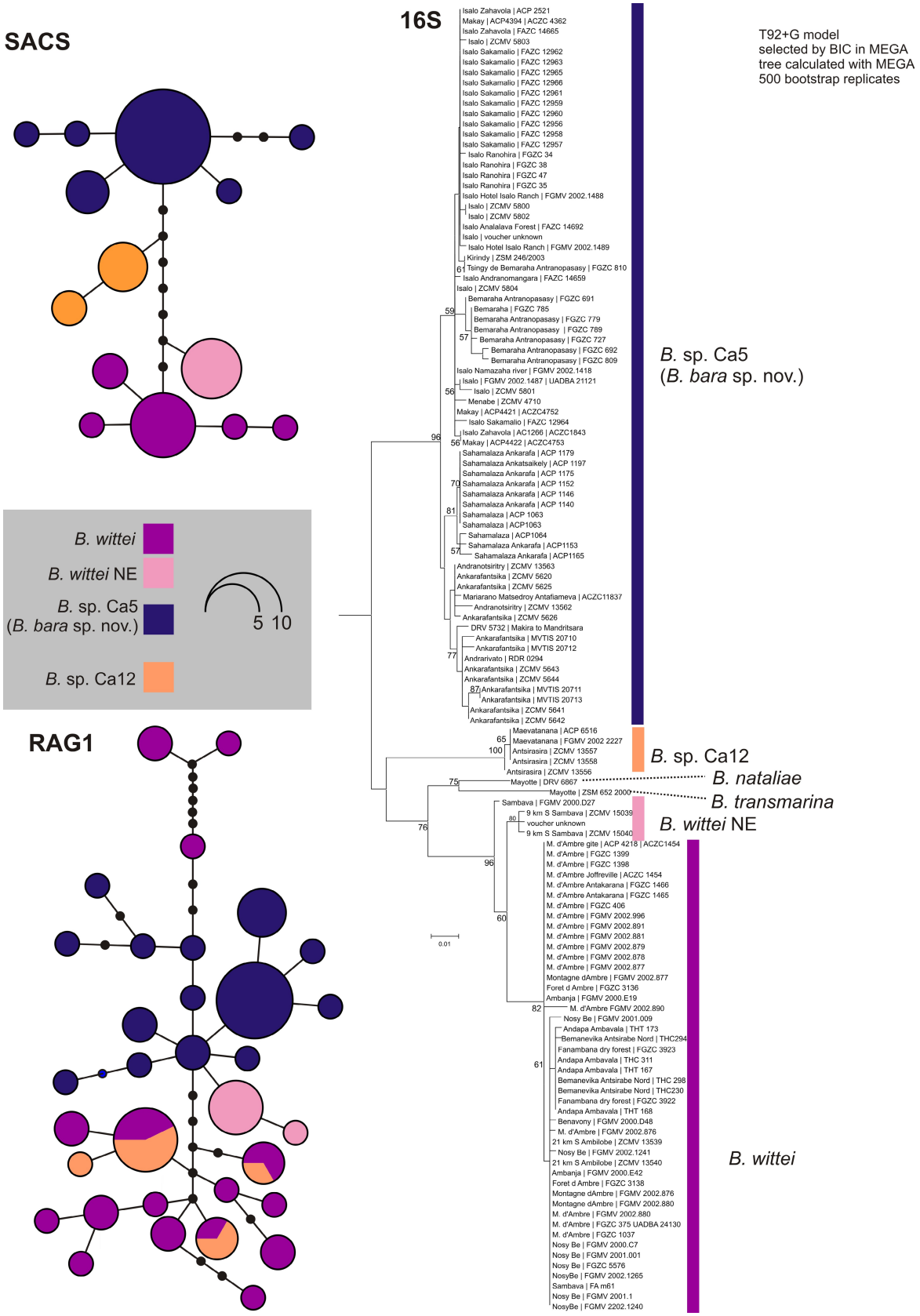

FIGURE 1. Molecular differentiation in the *Blommersia wittei* complex. The panels show a maximum likelihood tree based on 533 bp of the mitochondrial 16S tree (on the right), numbers at nodes representing bootstrap percentages (500 replicates). The two panels on the left show haplotype networks reconstructed from phased DNA sequences of fragments of the nuclear-encoded SACS (23 samples, 924 bp) and RAG1 (33 samples, 475 bp) genes. Colors in the network correspond to assignment of samples to main groups in the 16S tree.

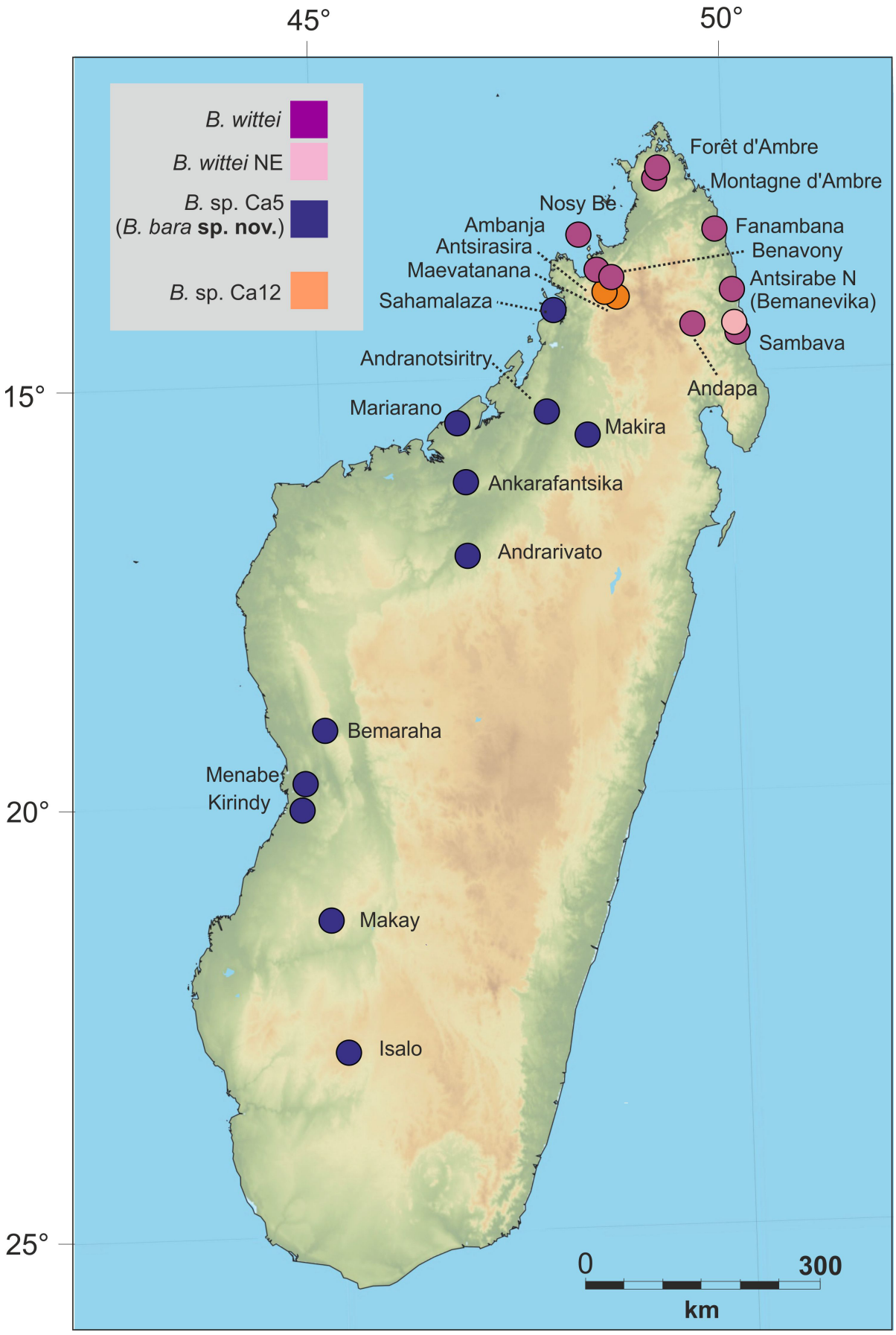

FIGURE 2. Map of Madagascar showing the distribution records of the *Blommersia wittei* species complex. Only locations confirmed by molecular data are shown.

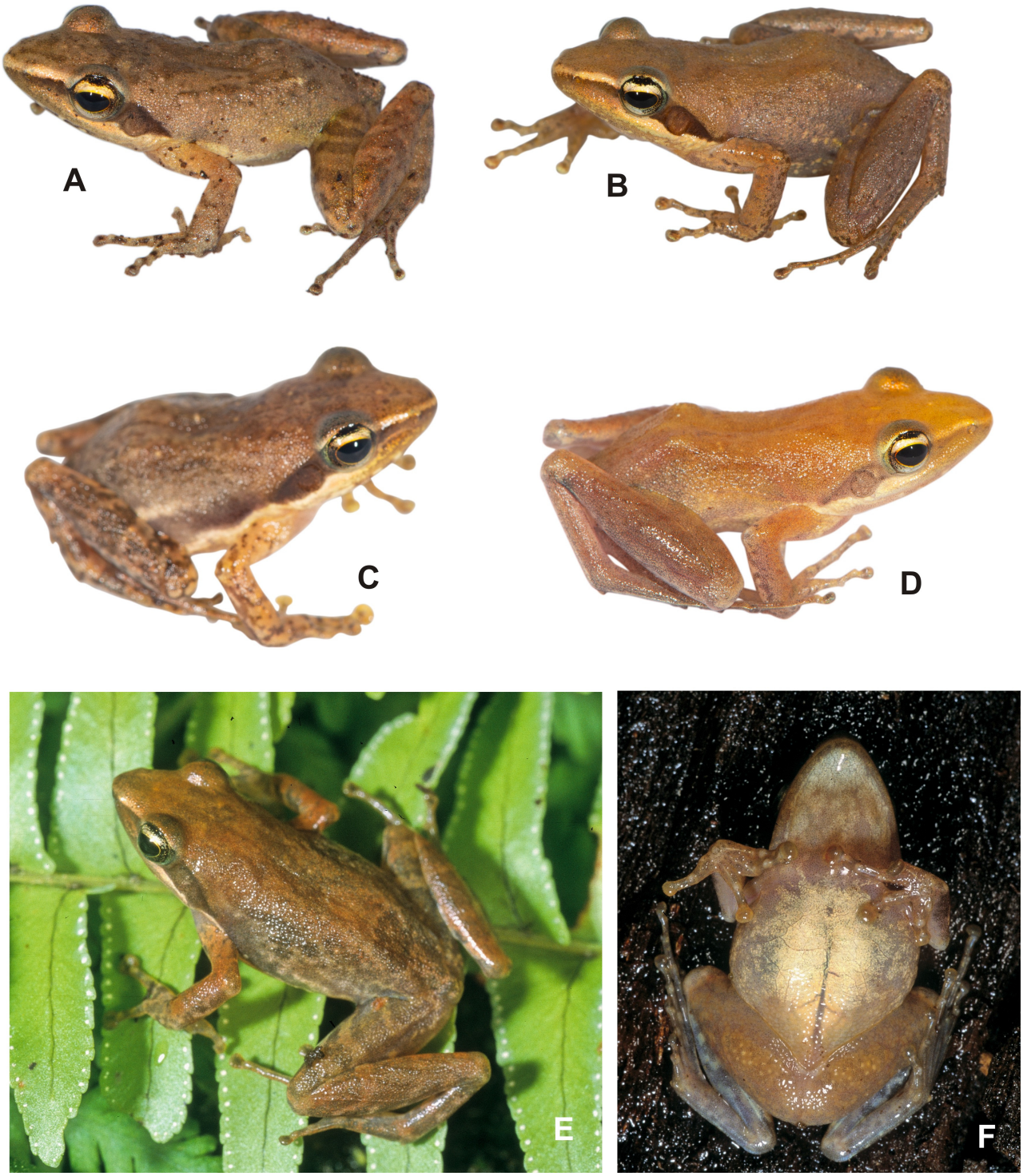

FIGURE 3. Specimens of *Blommersia wittei* in life. (A–C) Specimens UADBA-MSZC 451, ZSM 51/2018 (MSZC 404), ZSM 50/2018 (MSZC 521) from Montagne d'Ambre. (D) Specimen UADBA-FGZC 5576 from Nosy Be. (E,F) Male specimen from Montagne d'Ambre (not clearly referable to a voucher specimen) photographed in 2003.

Morphological differentiation

Morphological examination of available specimens revealed no consistent differences among the main lineages in the *B. wittei* complex (except for the distinct Comoran species that are not considered here). In contrast, a

substantial polymorphism in various traits was apparent (Figs 3–4). All specimens examined shared a separation of the lateral metatarsalia by webbing (rather than connective tissue) which is a diagnostic trait of *B. wittei* and a few other *Blommersia* species. Vomerine teeth were present in most specimens, but were sometimes weakly expressed (e.g., in *B. wittei* from Montagne d'Ambre) and in some specimens (like the small-sized specimens of *B.* sp. Ca5 from Isalo) not recognizable. Femoral glands were distinct in many male specimens, but indistinct in the sole male specimens available from Antsirasira and Sambava (Table 1). A very substantial difference was observed in body size, especially within *B.* sp. Ca5 where specimens from Isalo (male SVL 18.5–20.5 mm) were much smaller than those from Ankarafantsika and Tsingy de Bemaraha (23.4–25.7 mm) (Table 1). A thin middorsal line or a broad middorsal stripe characterize some specimens, but otherwise color and pattern was inconspicuous and rather uniform across specimens, populations and lineages.

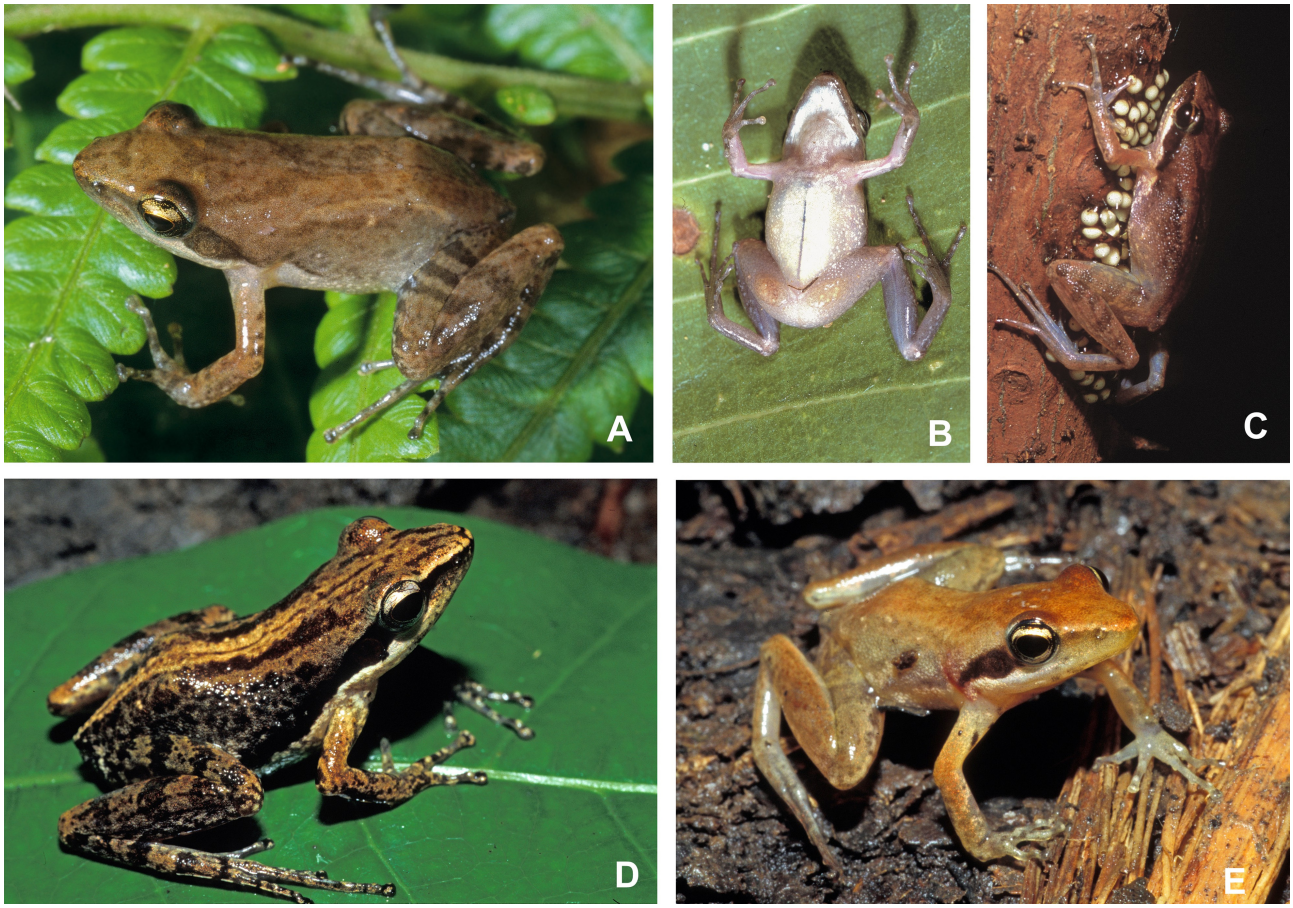

FIGURE 4. *Blommersia bara* **sp. nov.** (= *B.* sp. Ca5) in life. (A,B): Male holotype ZSM 31/2004 (FGZC 47) from Isalo in dorsolateral and ventral view. (C) Individual from Ankarafantsika displaying egg-guarding behavior. (D) Female paratype ZSM 13/2006 from Tsingy de Bemaraha. (E) Additional individual (not clearly referable to a voucher) from Tsingy de Bemaraha.

Bioacoustic differentiation

Advertisement calls within the *Blommersia wittei* complex all are characterized by consisting of a single short note emitted in call series at more or less regular intervals (Figs 5–7). Depending on motivation, call series might be of different duration and contain differing numbers of calls (Vences *et al.* 2006). Although this character is shared by all calls studied herein, there are some notable differences in note structure among the populations considered.

Calls of populations here referred to as corresponding to *B. wittei*, including those from the type locality Ambanja, differ distinctly in note structure from the other populations by notes composed of numerous pulses, mostly well separated from each other and grouped in two pulse groups within each note, separated by a longer inter-pulse interval (Fig. 5). In all cases, the first pulse group within a note exhibits less call energy than the second pulse group of the same note. Although our call recordings of *B. wittei* differ markedly in recording quality, this character is obvious in all *B. wittei* calls analyzed, except those from Sambava that are separately characterized below.

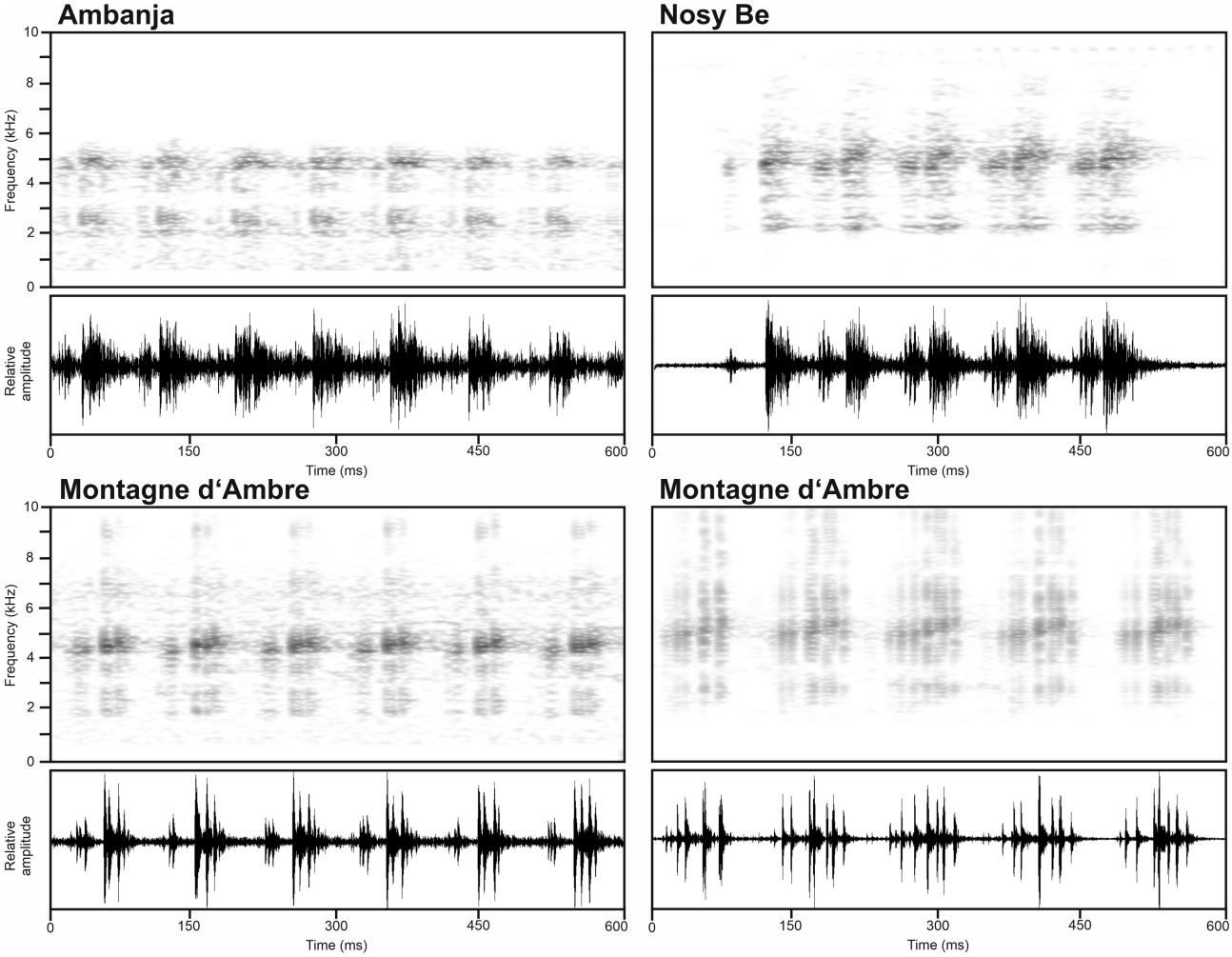

FIGURE 5. Audiospectrograms and corresponding oscillograms of advertisement calls of *Blommersia wittei* from three different localities (each showing several calls of a call series).

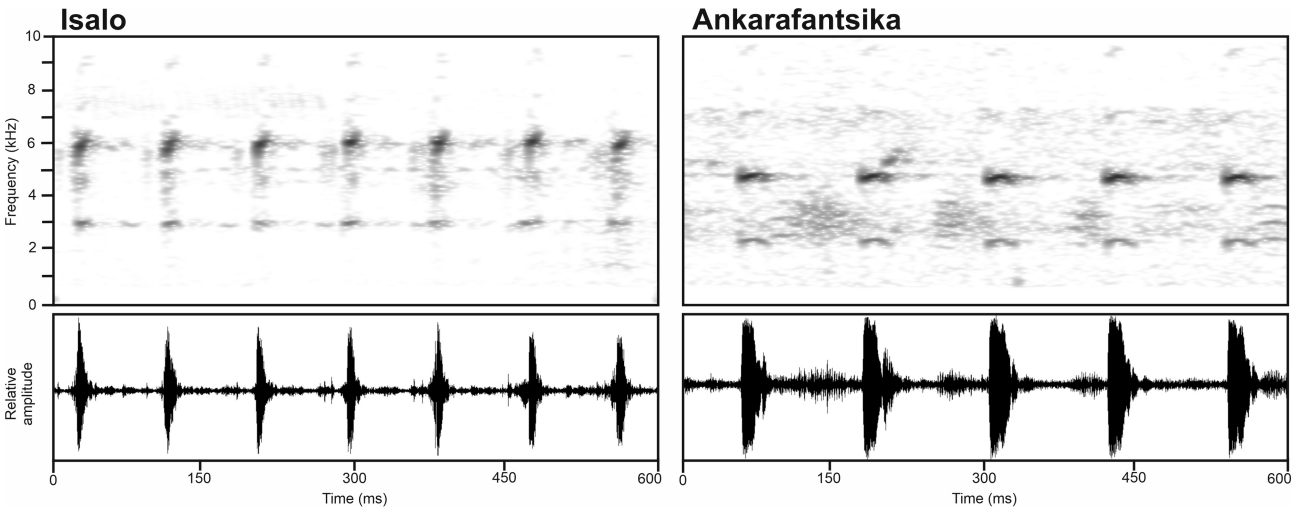

FIGURE 6. Audiospectrograms and corresponding oscillograms of advertisement calls of *Blommersia bara* **sp. nov.** (= *B.* sp. Ca5) from two different localities (each showing several calls of a call series).

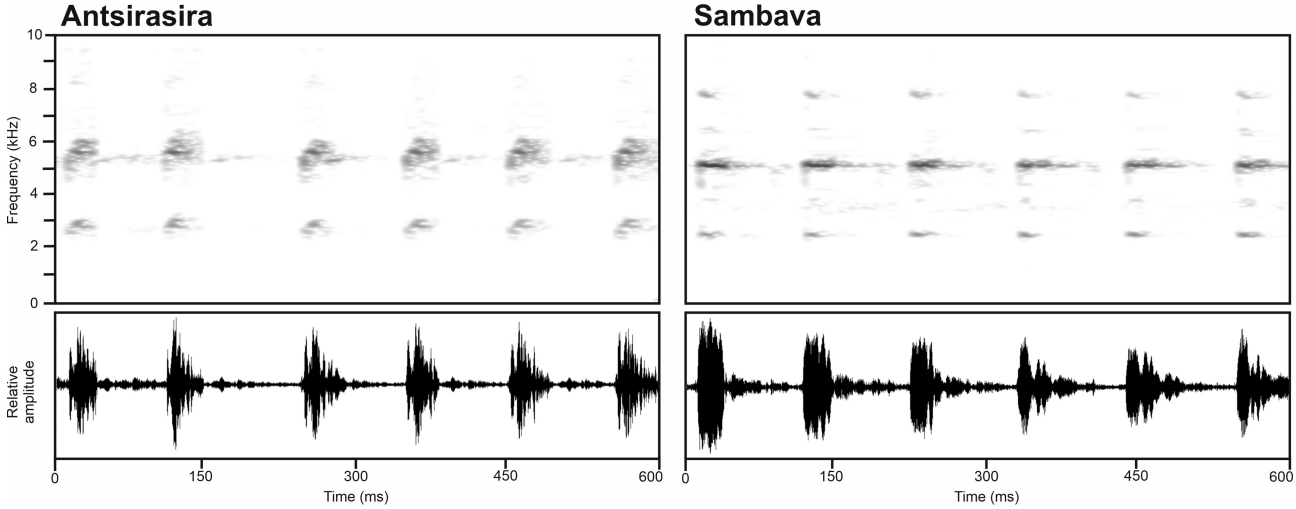

FIGURE 7. Audiospectrograms and corresponding oscillograms of advertisement calls of *Blommersia* populations of taxonomically uncertain status (each showing several calls of a call series). The recording from Antsirasira may refer to *B.* sp. Ca12, but the recorded specimen has not been collected or genotyped. The call from Sambava is from a specimen of *B. wittei* that belongs to a weakly divergent mitochondrial phylogroup and may also differ in some morphological features, but is here assigned to *B. wittei* preliminarily.

Populations from western Madagascar, here referred to as *B.* sp. Ca5, emit advertisement calls with notes being very short and barely structured with respect to amplitude modulation (Fig. 6). Calls from Isalo may contain 2–4 amplitude peaks, but are rather simple and homogeneous in structure. The same is true for calls from Ankarafantsika, with sometimes only two recognizable amplitude peaks within notes and only slightly longer note duration when compared to calls from Isalo. The differences among the calls of these two populations are rather small and would not qualify for species-specific differences (see Köhler *et al.* 2017), given their geographic distance to each other.

Calls recorded from Antsirasira (no voucher specimen collected from the calling site; calls perhaps corresponding to *B.* sp. Ca12) differ considerably from those of *B.* sp. Ca5 by a much more complex amplitude structure within notes (Fig. 7), with each note containing several separated pulses (3–10 pulses/note), which can be partly fused. Furthermore, note duration is considerably longer, when compared to calls from the dry west (34–66 versus 13–28 ms).

Calls from Sambava in the North-East (from a population here assigned to *B. wittei*) are rather similar when compared to calls from Antsirasira (*B.* sp. Ca12), as these share a similar pulse pattern and note duration (Fig. 7). However, pulses in Sambava calls were largely fused and the pulses in most notes are thus barely countable, and dominant frequency seems to be distributed in a narrower frequency band.

In summary, the bioacoustic data are in general agreement with our results of molecular phylogenetic analyses in supporting the distinctness of three Malagasy clades in this species complex, with advertisement calls showing characteristic traits for each identified clade. Although call differences are slightly less pronounced among these clades when compared to other groups in *Blommersia* (Vences *et al.* 2010; Pabijan *et al.* 2011), the bioacoustic differences identified do support our taxonomic conclusions.

Taxonomic conclusions

The genetic data of mitochondrial and nuclear-encoded DNA sequences suggest the existence of various species within the *B. wittei* complex. This is supported by deep mitochondrial divergences around 5% among the three main Malagasy mitochondrial lineages, reduced or absent haplotype sharing between these lineages in two nuclear-encoded gene fragments, bioacoustic differences between two of the lineages, and possibly by the phylogenetic placement of the Comoran clade of two morphologically and ecologically distinct species, *B. nataliae* and *B. transmarina*, nested among the Malagasy lineages.

Completely resolving this complex of species is, however, hampered by uncertainties in both, assignment of existing names and species delimitation. From a nomenclatural point of view, our data do not allow us to assign the available name, *B. wittei*, with full confidence to one of the lineages, as no molecular data are available from the *B. wittei* holotype. The closest occurrence of *B.* sp. Ca5, at Sahamalaza, is located at more than 70 km linear distance from the *B. wittei* type locality Ambanja, and we therefore consider it as unlikely that *B.* sp. Ca5 would occur up to Ambanja; its synonymy with *B. wittei* can therefore be excluded with sufficient probability. However, Ambanja is located along the lower Sambirano river and in this general area two deep lineages occur: the widespread northern lineage, which was found by us at Ambanja and nearby sites (Benavony, Nosy Be), and the deep microendemic lineage here named *Blommersia* sp. Ca12 occurring somewhat further upstream. We here consider to be most likely that the holotype and topotypical specimens of the paratype series are conspecific with the genetic lineage found by us in Ambanja. We cannot, however, fully rule out that also *B.* sp. Ca12 occurs at Ambanja, and that the holotype of *B. wittei* may belong to this lineage.

From the point of view of species delimitation, the status of the population from Sambava in the North East remains unclarified. This lineage has unique haplotypes in the two nuclear genes and differs in advertisement call, but exhibits only a weak mitochondrial divergence from typical *B. wittei* (ca. 1% 16S distance). Furthermore, one sample purportedly from Sambava has a mitochondrial sequence similar to that of typical *B. wittei*. However, this individual (voucher number FA m61, corresponding to the sequence with accession number AY848113 and catalogued under MRSN A3785) was collected in 2000 (by MV) without assigning it a physical fieldnumber tag, and we cannot exclude that the sample and/or voucher specimen has been incorrectly labelled as originating from Sambava. New, more comprehensive collections are needed to understand if specimens with the mitochondrial identity of typical *B. wittei* indeed occur in the Sambava region.

As a taxonomic conclusion, we suggest that the western/north-western lineage (*B.* sp. Ca5) represents a distinct species due to its genetic and bioacoustic divergence, and because its range is far enough from the *B. wittei* type locality (Ambanja) to exclude that the *B. wittei* holotype belongs to this lineage. We also conclude that the microendemic Sambirano lineage (*B.* sp. Ca12) very probably represents a distinct species which, however, we cannot formally name and describe at this time due to the lack of voucher specimens available for examination, the absence of the molecular identification of the B. wittei holotype, and the closeness of its range to the type locality of *B. wittei.* Finally, we do not formally name the Sambava population (neither as species, subspecies nor candidate species) due to its low mitochondrial divergence and uncertainty about its co-occurrence with typical *B. wittei*.

Species accounts

Blommersia wittei (Guibé, 1974)

Figures 3, 8

Identity and type material. Clarifying the taxonomy of the *B. wittei* complex requires first ascertaining the identity of *B. wittei* sensu stricto. The species was described by Guibé (1974) as *Mantidactylus wittei*, and type specimens are deposited in the Paris museum (acronym MNHN, in earlier times written MNHNP), with the following verbatim information in the original description: "*Holotype : n° 1953-60 MNHN Paris. Environs d'Ambanja. J. Guibé (11-1951). Paratypes : n^os^ 1953-60 A à 1953-60 L MNHN Paris. Même provenance. N^os^ A.682 à A.684 : Ampijora, station forestière à 45 km de Marovaoy (Ouest). N^os^ 1973-944 à 1973-951 MNHN Paris : forêt d'Ankarafantsika. Ch. P. Blanc (11-1973).*"

Due to some imprecise information in the handwritten MNHN catalogue and extensive re-labelling of several of these specimens, there has been confusion on the type series in subsequent publications and in the current digital MNHN catalogue. Frost (2023) correctly states that the holotype is MNHNP 1953.60, by original designation, but merges two separate sites in the type locality account: "*'Environs d'Ambanja'*, *Forest Ankarafantsika*, *Madagascar.*"

In contrast, the digital type catalogue of the MNHN (downloaded from gbif.org in 2022) as well as the MNHN online catalogue (https://science.mnhn.fr/institution/mnhn/collection/ra/; accessed 11 February 2023) state that the holotype originated from "*Vallée de la Tsiribihina*" and furthermore provide the following list of paratypes: MNHN 1973.944–1973.951 (8 specimens) from Ankarafantsika, and 1993.682–1993.684 (3 specimens) from Ampijoroa.

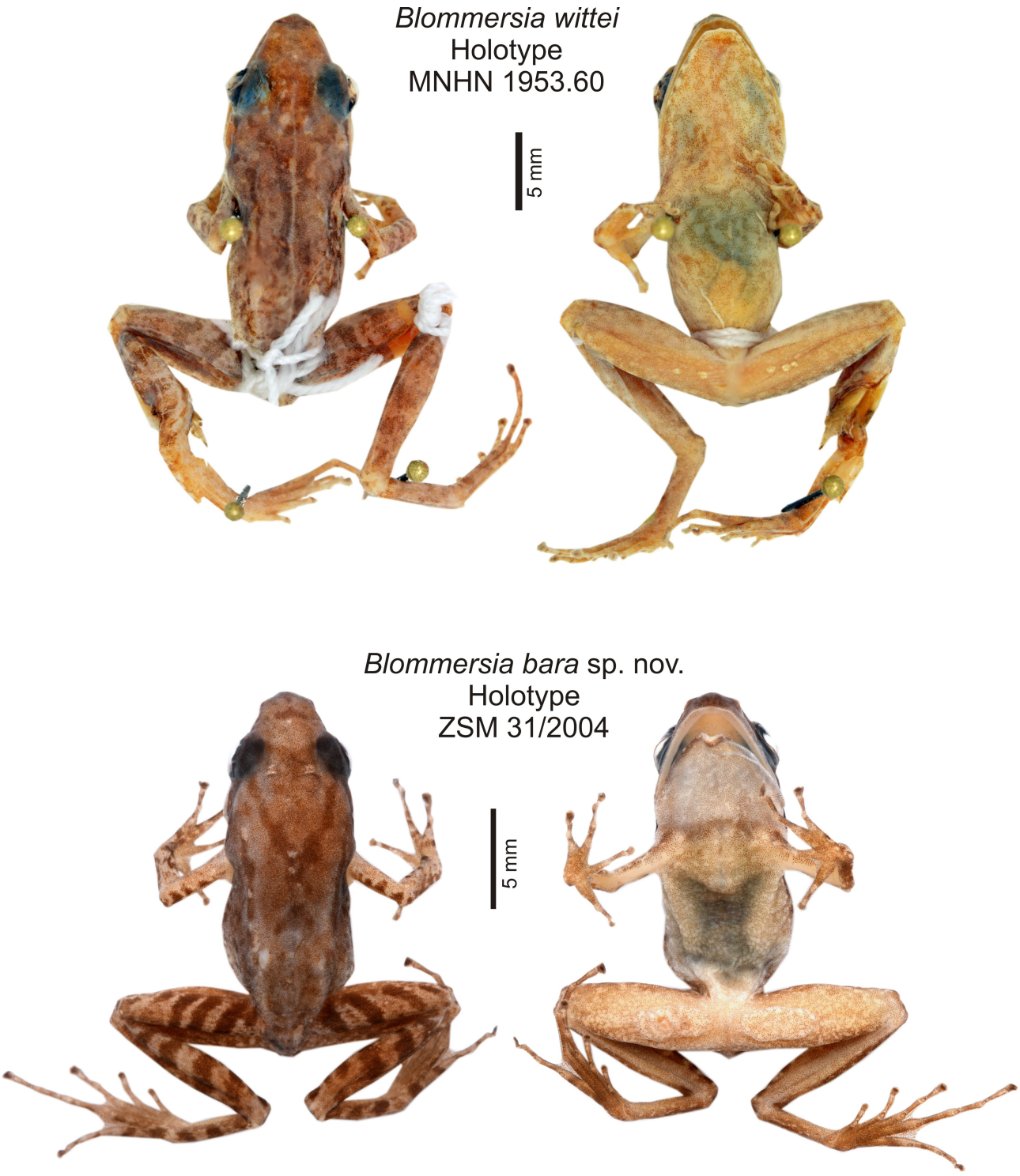

FIGURE 8. Preserved name-bearing type specimens of *Blommersia wittei* and *Blommersia bara* **sp. nov.** in dorsal and ventral views. Photographs of the *B. wittei* holotype by RECOLNAT (ANR-11-INBS-0004) / Antoine Fraysse, available from https://science.mnhn.fr/institution/mnhn/collection/ra/item/1953.60.

Vences *et al.* (2010) provided measurements of part of the type series of *B. wittei*, purportedly all from Ambanja: the holotype MNHN 1953.60, and the following paratypes: MNHN 1991.2529–1991.2533 (5 specimens; previously labelled MNHN 1953.60 A to E) and MNHN 1991.2536–1991.2539 (4 specimens, previously labelled MNHN 1953.60 H to K).

After inspecting the original (handwritten) MNHN catalogues (partly available from https://science.mnhn.fr/catalogues; accessed 11 February 2023) we conclude that:

(1) The holotype of *Mantidactylus wittei* Guibé, 1974 (Fig. 8) unambiguously is the specimen MNHN 1953.60 for which morphometric measurements were provided by Vences *et al.* (2010) and whose locality, according to the original description and original catalogue entry is "*Environs d'Ambanja"*, with the addition in the catalogue: *"Cacaoyéres"* (= cacao trees, thus indicating the specimen was collected in a cacao plantation).

(2) A series of 11 paratypes (originally labelled MNHN 1953.60 A to 1953 K, currently re-labelled as MNHN 1991.2529–1991.2539) originates from the same locality as the holotype.

(3) A series of 8 paratypes (MNHN 1973.944–1973.951) originate from Ankarafantsika,

(4) A series of 3 paratypes (MNHN 1993.682–1993.684) originate from Ampijoroa, which represents a forestry station (currently local headquarters of Madagascar National Parks) within the Ankarafantsika forest.

As explained in the Taxonomic conclusion account above, based on the provenance of the holotype from Ambanja, we continue assigning the name *B. wittei* to the genetic lineage occurring across much of northern Madagascar and collected by us at Ambanja and nearby localities. The paratype series is however mixed: specimens from Ankarafantsika (MNHN 1973.944–1973.951) and Ampijoroa (MNHN 1993.682–1993.684) are here assigned to *B.* sp. Ca5. Only the 11 paratypes from the type locality are likely conspecific with the holotype.

Morphology. Measurements of the type series of *B. wittei* have been published by Vences *et al.* (2010) and measurements of additional specimens by Pabijan *et al.* (2011). Measurements of further specimens are included in Table 1 herein. A full set of measurements including those from the previous publications is given as Supplementary Table 2 (available from the Zenodo repository under DOI 10.5281/zenodo.8049142). Based on these data, male SVL is 21.0–27.0 mm and female SVL is 20.7–25.0 mm. In the specimens examined for the present study (listed in Table 1), vomerine teeth are usually recognizable (clearly visible in specimens from the type locality Ambanja) but weakly expressed in several specimens from Montagne d'Ambre. In ZSM 563/2000 from Sambava, vomerine teeth are weakly recognizable, and this specimen apparently has more developed webbing than many other individuals examined.

Vocalizations. Advertisement calls recorded in February 1991 at the type locality Ambanja (recording temperature not taken) consist of a single short pulsed note repeated in call series at regular intervals and very fast succession (Fig. 5). Recording quality is poor and the detailed call structure is probably partly masked by background noises. However, each call (= note) seems to contain two pulse groups which are separated from each other, with the first pulse group being of lower amplitude. Pulse structure in not very obvious in the recording and most pulses appear basally fused. Maximum call energy is distributed towards the middle of the call's duration. Numerical parameters of 16 analyzed calls of one male are as follows: call duration (= note duration) 62–77 ms (66.8 ± 5.3 ms); inter-call intervals within regular call series 15–31 ms (18.6 ± 5.6 ms); pulses/note 9–18 (13.8 ± 3.3); duration of regular call series 1275 ms (n = 1); call rate within call series approximately 740 calls/minute; dominant frequency 4694–4886 Hz (4797 ± 82 Hz); second frequency peak around 2500 Hz; prevalent bandwidth 1800–6000 Hz.

Advertisement calls recorded on 7 February 1992 from north of Andoany, Nosy Be (air temperature 25°C), consist of a single short pulsed note repeated in short call series at regular intervals and very fast succession (Fig. 5). Each call (= note) exhibits two pulse groups which are clearly separated from each other, with the first pulse group being of lower amplitude. Pulses are partly fused, but countable. Maximum call energy is distributed in the middle of the call, namely the beginning of the second pulse group. Numerical parameters of 10 analyzed calls of one male are as follows: call duration (= note duration) 65–83 ms (74.8 ± 6.3 ms); inter-call intervals within regular call series 16–32 ms (21.5 ± 5.6 ms); pulses/note 14–19 (16.3 ± 1.9); duration of regular call series 490 and 500 ms (n = 2); call rate within call series approximately 640 calls/minute; dominant frequency 4823–5240 Hz (5033 ± 163 Hz); second frequency peak around 2500 Hz; prevalent bandwidth 2000–8200 Hz.

Advertisement calls recorded on 15 March 1994 on Montagne d'Ambre (air temperature 22°C), consist of a single short, distinctly pulsed note repeated in call series at regular intervals and very fast succession (Fig. 5). Each call (= note) exhibits two pulse groups which are clearly separated from each other, with the first pulse group being of lower amplitude. Pulses are barely fused and rather distinctly separated. Maximum call energy is distributed in the middle of the call, namely the beginning of the second pulse group. Numerical parameters of 19 analyzed calls of one individual are as follows: call duration (= note duration) 59–73 ms (65.4 ± 4.2 ms); inter-call intervals within regular call series 22–39 ms (29.6 ± 7.1 ms); pulses/note 7–11 (8.9 ± 1.3); duration of regular call series 1821 ms (n = 1); call rate within call series approximately 600 calls/minute; dominant frequency 4489–4597 Hz (4542 ± 41 Hz); prevalent bandwidth 1800–7500 Hz.

Advertisement calls recorded from specimen ZSM 50/2018 (MSZC 521) on 25 December 2017 on Montagne d'Ambre (air temperature ca 19°C), consist of a single short, distinctly pulsed note repeated in call series at regular intervals and very fast succession (Fig. 5). Each call (= note) exhibits clearly separated pulses. Intervals between pulses are somewhat irregular, in some calls resulting in two pulse groups separated by a larger interval. Maximum call energy is evident in the middle of the call. Numerical parameters of 16 analyzed calls of one male are as follows: call duration (= note duration) 68–88 ms (78.2 ± 5.7 ms); inter-call intervals within regular call series 28–50 ms (41.4 ± 4.2 ms); pulses/note 7–11 (9.0 ± 1.3); duration of regular call series 1590 ms (n = 1); call rate within call series approximately 500 calls/minute; dominant frequency 5066–5232 Hz (5137 ± 80 Hz); prevalent bandwidth 2000–10000 Hz.

Advertisement calls recorded on 20 March 2000 in Sambava (air temperature 24.7°C), here tentatively allocated to *B. wittei*, consist of a single, short, pulsed note repeated in call series at regular intervals within series. Calls (= notes) exhibit a pulsed structure, but most pulses are largely fused, resulting in an irregular pulse pattern and varying number of countable pulses (Fig. 7). Maximum call energy is distributed among the first two thirds of the call's duration. Numerical parameters of 22 analyzed calls of one male are as follows: call duration (= note duration) 27–59 ms (39.4 ± 11.8 ms); inter-call intervals within regular call series 54–76 ms (67.9 ± 7.7 ms); pulses/note 2–6 (4.1 ± 1.0); duration of regular call series 2209 ms (n = 1); call rate within call series approximately 600 calls/minute; dominant frequency 5316–5598 Hz (5440 ± 145 Hz); second frequency peak around 2600 Hz; prevalent bandwidth 2100–8600 Hz.

Blommersia bara **sp. nov.**
Figures 4, 8

Identity. This species was considered *Mantidactylus wittei* by Guibé (1974) and Blommers-Schlösser & Blanc (1991) for specimens from Ankarafantsika, and called *Mantidactylus* cf. *wittei* by Glos (2003), *Blommersia* sp. aff. *wittei* "Isalo" by Glaw & Vences (2007), *Blommersia* sp. aff. *wittei* by Mercurio *et al.* (2008), *Blommersia* sp. (aff. *wittei*) by Bora *et al.* (2010), *Blommersia* sp. 5 by Vieites *et al.* (2009) and Wollenberg *et al.* (2011), *Blommersia* sp. Ca5 by Perl *et al.* (2014), Penny *et al.* (2016) and Glaw *et al.* (2019), *Blommersia* sp. Ca05 by Penny *et al.* (2017), and *Blommersia* sp. aff. *wittei* Ca05 "Isalo" by Cocca *et al.* (2018).

Holotype. ZSM 31/2004 (field number FGZC 47), an adult male, collected by F. Glaw, M. Puente, M. Thomas & R. Randrianiaina at a stream near Ranohira, Isalo Massif (22.5856°S, 45.3997°E, 813 a.s.l.), southwestern Madagascar, on 21 January 2004.

Paratypes. A total of 30 specimens, all from western Madagascar. ZSM 22/2004 (FGZC 34) and ZSM 24/2004 (FGZC 38) two adult males with the same collecting data as the holotype; ZSM 706/2001 (FGMV 2001.279), adult male, collected by M. Vences, D.R. Vieites, G. Garcia, V. Raherisoa, & A. Rasoamamonjinirina at Ampijoroa, Ankarafantsika National Park (approximately 16.3°S, 46.82°E) on 24 February 2001; ZSM 246/2003 and ZSM 247/2003, two adult males, collected by J. Glos at Kirindy Forest (site "G2") in January 2001; ZSM 13/2006 (FGZC 691), ZSM 30/2006 (FGZC 727), ZSM 52/2006 (FGZC 779), and ZSM 68/2006 (FGZC 809), two adult females and two adult males, collected by F. Glaw, J. Köhler, P. Bora & H. Enting at Tsingy de Bemaraha National Park, Andranopasazy, "Camp 1" (18.7086°S, 44.7189°E, 146 m a.s.l.), on 17, 19, and 24 March 2006, respectively; ZSM 2284/2007 (ZCMV 5801) and ZSM 2285/2007 (ZCMV 5804), one adult male and one adult female, collected by M. Vences and collaborators at Isalo National Park, on 17 February 2007; ZSM 2281/2007 (ZCMV 5625), ZSM 2282/2007 (ZCMV 5626), ZSM 2283/2007 (ZCMV 5643), and ZSM 2320/2007 (ZCMV 5621), collected by L. du Preez, C. Weldon & L. Raharivololoniaina at Ankarafantsika, on 9 February 2007; ZSM 3222/2012 (ZCMV 14143), adult male collected by A. Rakotoarison, J. Erens & E. Rajeriarison at Mariarano (15.4978°S, 46.6943°E, 11 m a.s.l.), on 28 December 2012; MRSN A2957 and A2958 (FAZC 11807–11808), two males, collected by F. Andreone, G. Aprea & V. Mercurio in the Isalo Massif, Andohasahenina (22.8333°S, 45.1880°E, 876 m a.s.l.) on 15 January 2004; MRSN A5349 (FAZC 12550), female, and MRSN A5350 (FAZC 12551), male, collected by F. Andreone, F. Mattioli & V. Mercurio in the Isalo Massif, Zahavola (22.6215°S, 45.3587°E, 881 m a.s.l.), on 17 November 2004; MRSN A5353 and A5354 (FAZC 12968 and 12970), two males, collected by F. Andreone, F. Mattioli & V. Mercurio in the Isalo Massif, Sakamalio (22.4348°S, 45.2552°E, 726 m a.s.l.) on 16 December 2004; MRSN A5351 (FAZC 12591), female, collected by F. Andreone, F. Mattioli & V. Mercurio in the Isalo Massif,

Andriamanero (22.3672°S, 45.3920°E, 663 m a.s.l.), on 20 November 2004; as well as seven specimens deposited in the UADBA collection (uncatalogued but accessible via field tags): UADBA-ZCMV 5620, 5641, 5642, 5644, four specimens collected by L. du Preez, C. Weldon & L. Raharivololoniaina at Ankarafantsika, on 9 February 2007; and UADBA-ZCMV 5800, 5802, 5803, three specimens collected by collected by M. Vences and collaborators at Isalo National Park, on 17 February 2007.

Diagnosis. A species of the genus *Blommersia* in the subfamily Mantellinae of the family Mantellidae based on presence of intercalary elements between penultimate and ultimate phalanges of fingers and toes (verified by external examination), occurrence in Madagascar, relatively small body size (male SVL < 27 mm), presence of femoral glands in males and absence of femoral gland rudiments in females, head distinctly longer than wide, and molecular phylogenetic relationships.

From other species of *Blommersia*, the new species is mainly distinguished as follows: from *B. angolafa* by the presence of vomerine teeth in many individuals (vs. absence) and a totally different color pattern without whitish spots on the flanks and on the finger- and toe-tips (vs. presence); from *B. dejongi* by a femoral gland placed centrally on the thigh (vs. distally); from *B. blommersae* by having lateral metatarsalia separated by webbing (vs. connected by dense tissue); from *B. domerguei* by having lateral metatarsalia separated by webbing (vs. connected by dense tissue), by the presence of vomerine teeth in many individuals (vs. absence), a larger body size (SVL >20 mm vs. <18 mm), and absence of a distinct pattern with three dark brown bands on a copper-brown dorsum (vs. presence); from *B. dupreezi* by having lateral metatarsalia separated by webbing (vs. connected by dense tissue) and absence of distinct black lateral stripe (vs. presence); from *B. galani* by presence of vomerine teeth in many individuals (vs. absence); from *B. grandisonae* by presence of vomerine teeth in many individuals (vs. absence) and a largely different color pattern (but see discussion in Vences *et al.* 2023 on the identity of *B. grandisonae*); from *B. kely* by having lateral metatarsalia separated by webbing (vs. connected by dense tissue), presence of vomerine teeth in many individuals (vs. absence), and larger body size (SVL >20 mm vs. <17 mm); from *B. nataliae* by ovoid femoral glands in small distance to each other (vs. more rounded, distant glands) and absence of a dark face mask (vs. presence); from *B. sarotra* by having lateral metatarsalia separated by webbing (vs. connected by dense tissue); from *B. transmarina* by a narrower head (male HW/SVL ratio 0.29–0.34 vs. 0.34–0.36) and somewhat shorter hands (male HAL/SVL ratio 0.29–0.33 vs. 0.30–0.35); from *B. variabilis* by having lateral metatarsalia separated by webbing (vs. connected by dense tissue in most individuals) and probably a smaller distance between femoral gland. Furthermore, differentiated from all these species by differences in advertisement calls and genetic distances >5% in the analyzed mitochondrial 16S rRNA gene fragment of these two species. It differs from *B. wittei*, however, in a substantial divergence of mitochondrial genes (5.0–8.2% uncorrected pairwise distance in 16S) and lack of haplotype sharing in RAG1 and SACS, and in advertisement calls lacking distinct pulses vs. being clearly pulsed in *B. wittei* (except in the Sambava population whose taxonomic status is in need of revision).

Description of the holotype. Adult male specimen with distinct femoral glands, in a good state of preservation (Fig. 8). Tongue removed as tissue samples for molecular analysis. SVL 18.5 mm, for further measurements see Table 1. Body slender; head longer than wide, of same width as body; snout rounded in lateral view, obtusely pointed in dorsal and ventral views; nostrils directed laterally, protuberant, nearer to snout tip than to eye; canthus rostralis rounded; loreal region slightly concave; tympanum distinct, round, its diameter 63% of eye diameter; supratympanic fold distinct, curved above tympanum where it follows the tympanum outline; tongue absent, its shape therefore not ascertainable; vomerine teeth not visible; choanae small, round, located toward the front of the palate; maxillary teeth present. Arms slender, subarticular tubercles present, single; fingers without webbing; relative length of fingers 1<2<4<3, finger discs enlarged, nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches center of eye when the hind limb is adpressed along the body; lateral metatarsalia entirely separated by webbing; comparatively small inner and much smaller outer metatarsal tubercles, which are present but not very distinct; webbing formula 1(traces), 2i(traces), 2e(1), 3i(2.5), 3e(1.75), 4i(2.75), 4e(3), 5(1.75); relative length of toes 1<2<5<3<4. Skin on the upper surface smooth, without folds or ridges. Ventral skin smooth to slightly shagreened. Femoral glands distinct and prominent.

After 19 years in preservative (Fig. 8), the dorsum is rather uniformly brownish, with a weakly recognizable dark brownish inverted chevron, a faint small dark transverse bar on the forehead anterior to the eyes, and a very weakly recognizable dark interorbital bar. A dark stripe runs from the nostril to the eye and continues broader posterior to the eye, encompassing the entire tympanic region underneath the supratympanic fold. Underneath these brown elements, a weakly expressed light frenal stripe is visible from the snout tip to the forelimb insertions. Fore-

and hindlimbs dorsally with distinct and sharply delimited brown crossbars (three crossbars on thigh and four on shank). Ventral side uniformly beige (unpigmented), throat being a bit lighter than chest, belly and limbs. In life, color was very similar but somewhat more contrasted (Fig. 4A); whitish color was present on the throat; femoral glands granules were somewhat yellowish (Fig. 4B).

Variation. ZSM 52/2006 exhibits a broad middorsal light band. Vomerine teeth are clearly visible in paratype ZSM 2281/2007 from Ankarafantsika, also recognizable (although weakly expressed) in the paratypes from Bemaraha. We observed substantial differences in body size among populations, with individuals from the type locality Isalo being distinctly smaller than those from other sites. According to available data (Supplementary Table 2; available at DOI 10.5281/zenodo.8049142) male SVL across all populations ranges between 18.2–25.7 mm and female SVL between 22.0–26.4 mm.

Etymology. Named after the Bara people, the ethnic group living in the area of Madagascar that includes the type locality of the new species, the Isalo Massif. The name is used as a noun in apposition.

Natural history. A relatively common species in Madagascar, living in areas of a certain humidity such as streams or swamps, including areas of secondary vegetation. During the rainy season, males call during day and night from the low vegetation of these water bodies. In Isalo this species is commonly found on the ground of gallery forests (e.g., Andriamanero, Sakamalio), along temporary rivers and in *Pandanus* swamps in the open savannah (e.g., Ilakaka, Zahavola). Tadpoles develop in temporary ponds and individuals of this species have been observed in egg-guarding behaviour both in Ankarafantsika (Fig. 4C) and Isalo (Mercurio *et al.* (2008). In the Sahamalaza Peninsula, this species seems to be abundant and it is found along streams and ponds in intact forested areas as well as in paddy fields in cleared areas. In Bemaraha, specimens were found in the leaf litter. In Kirindy, Glos (2003) found the species in the largest out of 200 ponds studied, and observed males calling at night from a bush at the water edge between 1–4 m above ground.

Vocalizations: Advertisement calls recorded on 29 January 1994 at Isalo (air temperature 21.5°C) consist of a single short pulsatile note repeated in call series at rather regular intervals within series (Fig. 6). Each call exhibits some irregular amplitude modulation, sometimes with 2–4 peaks recognizable within each call, with the second peak usually having the highest energy. Numerical parameters of 23 analyzed calls of one male are as follows: call duration (= note duration) 13–18 ms (15.2 ± 1.6 ms); inter-call intervals within regular call series 69–78 ms (74.8 ± 3.0 ms); duration of regular call series 2034 ms (n = 1); call rate within call series approximately 670 calls/minute; dominant frequency 5813–6115 Hz (5968 ± 119 Hz); second dominant frequency peak around 3000 Hz; prevalent bandwidth 2600–9500 Hz.

Advertisement calls recorded on 24 February 2001 at Ankarafantsika (air temperature 28°C) consist of a single short pulsatile note repeated in call series at regular intervals within series (Fig. 6). In some calls (= notes) two barely separated peaks of amplitude are recognizable, but call energy is distributed rather equally along the first two thirds of the call's duration, then dropping rapidly towards its end. Numerical parameters of 18 analyzed calls of one male are as follows: call duration (= note duration) 19–28 ms (23.2 ± 2.5 ms); inter-call intervals within regular call series 94–107 ms (97.3 ± 4.8 ms); duration of regular call series 2078 ms (n = 1); call rate within call series approximately 500 calls/minute; dominant frequency 4694–4758 Hz (4735 ± 27 Hz); second frequency peak around 2370 Hz; prevalent bandwidth 1900–9700 Hz.

Distribution. Known from Isalo in the South-West up to Sahamalaza Peninsula in the North-West (see Fig. 2 for genetically confirmed sites). The precise contact zone with *B. wittei* has not yet been identified but can be expected to be located between Sahamalaza and Ambanja.

Blommersia sp. Ca12

Identity. This candidate species name refers to a new lineage discovered in the course of this study and found only at two sites along the Sambirano river (Maevatanana and Antsirasira).

Morphology. Unknown. One voucher specimen from Antsirasira (ZSM 589/2001; see Table 1) could not be genotyped as no tissue sample for molecular analysis was taken in the field from this specimen. This specimen is an adult male with rather indistinct femoral glands and weakly expressed vomerine teeth. Photos in life are not available.

Vocalizations: Advertisement calls were recorded on 31 January 2001 at Antsirasira (air temperature 26°C), but

call vouchers were not collected and calls are therefore not assignable to *B.* sp. Ca12 with full reliability. These calls consist of a single, short, pulsed note repeated in call series at slightly irregular intervals within series (Fig. 7). Calls (= notes) generally exhibit a pulse structure, although some pulses are not clearly separated but rather largely fused, resulting in an irregular pulse pattern and varying number of countable pulses. Numerical parameters of 10 analyzed calls of one male are as follows: call duration (= note duration) 34–66 ms (47.8 ± 10.8 ms); inter-call intervals within regular call series 48–72 ms (61.4 ± 10.2 ms); pulses/note 3–10 (6.9 ± 2.3); duration of regular call series 1434 and 995 ms (n = 2); call rate within call series varied between 500–580 calls/minute; dominant frequency 5316–5598 Hz (5440 ± 145 Hz); second frequency peak around 2850 Hz; prevalent bandwidth 2200–9000 Hz.

Discussion

The initial goal of this study was to clarify the taxonomic status of the candidate species *Blommersia* sp. Ca5 which had been identified over 15 years ago based on its bioacoustic and strong genetic divergence from *B. wittei* (Glaw & Vences 2007; Vieites *et al.* 2009). Unexpectedly, however, the taxonomy within the *B. wittei*-complex turned out to be more convoluted than initially thought. We found the new species, herein formally named *B. bara*, occurring not only in the West (Isalo, Makay and Bemaraha) but also in several localities in the North West of Madagascar, including Ankarafantsika, which previously was thought to be inhabited by *B. wittei* (Glaw & Vences 2007) and which is the origin of several paratypes of that species. We found *B. wittei* to be somewhat genetically heterogeneous, with the population from the North East (Sambava) differing by a pairwise 16S distance of 1.2%, and distinct also in bioacoustics (less pulsatile call structure) from other populations. Most importantly, however, we discovered a previously unnoticed deep genetic lineage in the Sambirano valley, here named *B.* sp. Ca12, that differed both by deep genetic divergence in 16S (up to 7% uncorrected genetic distance to *B. wittei*) and by very reduced allele sharing in nuclear genes even from neighboring populations of *B. wittei*. Taken together, the biogeographic pattern in the *B. wittei* complex likely reflects the complexity of speciation mechanisms in the Malagasy herpetofauna (Brown *et al.* 2014): with a widespread species in the West and North West geographical regions (*B. bara*), a widespread species in the Sambirano, North and North East regions containing at least one deep genetic lineage (*B. wittei*), two species from the Comoran island of Mayotte that likely speciated in sympatry on this rather small island (Glaw *et al.* 2019; Vieites *et al.* 2020), and one microendemic species only known from lowlands along the Sambirano river (*B.* sp. Ca12).

Western Madagascar is characterized by relatively arid and highly seasonal climate (Jury 2022). Such conditions could be hypothesized to be unsuitable for small, partially arboreal frogs which therefore would be restricted to relict areas of more humid conditions e.g., in isolated massifs or in river gallery forests. However, our analysis revealed low and poorly structured mitochondrial divergence in *B. bara* across a wide range from Isalo to Sahamalaza, at an aerial distance of about 1000 km. The 16S distances between the main phylogroups of *B. bara* were at 1.6% or below, which is only slightly more than the distances observed among populations of the larger-sized co-distributed species *Laliostoma labrosum* (up to 1.1%; Pabijan *et al.* 2015), but considerably more than in *Boophis doulioti* (<1%; Vences & Glaw 2002). Interestingly, despite this relatively weak genetic differentiation among *B. bara* phylogroups, a quite substantial size difference was observed between *B. bara* individuals from Isalo vs. other sites. No obvious reasons for these size differences are apparent, but they align with the situation in at least one other frog species (*Boophis obscurus*) where Isalo populations are characterized by a distinctly smaller body size than those occurring in rainforest (Glaw *et al.* 2010). To understand whether these are genetically fixed differences in maximum size, or effects of e.g. timing of reproduction, speed of larval development, or resource availability, more in-depth studies of the natural history of these populations is necessary. If, for instance, in one population specimens metamorphose later or at smaller sizes at the end of the wet season than in other populations, these specimens will have less time for growing until the onset of the subsequent reproductive season and thus reproduce at smaller sizes (Scherz *et al.* 2023).

According to our revision, *Blommersia wittei* occurs in the Sambirano region, in the North, and North East regions of Madagascar, but its populations show some differentiation across its range. For example, specimens from Montagne d'Ambre appear to have more weakly expressed vomerine teeth than those from Ambanja, and especially, specimens from Sambava differ by bioacoustics, a mitochondrial genetic divergence of 1.2%, and lack of haplotype sharing in SACS and RAG1. The sole voucher specimen from this site also has poorly expressed femoral glands

and more extended webbing than voucher specimens from other sites. These differences may indicate taxonomic distinctness of these populations, but current data are insufficient for final conclusions: (i) the observed mitochondrial divergence is distinctly below the 3% used as a yardstick by Vieites *et al.* (2009) to define candidate species, and (ii) there is no call voucher that could reliably link bioacoustics, genetics and morphology of the Sambava population. Future material from Sambava and a fine-scale sampling of possible contact zones, e.g., northwards towards Montagne d'Ambre, and eastwards towards the Sambirano region, are necessary to comprehensively understand the level of differentiation within *B. wittei.*

The species status of *Blommersia bara* is obvious due to the concordance of genetic and bioacoustic divergence to its morphologically and phylogenetically closest congener on Madagascar, *B. wittei.* The diagnostic advertisement call structure of the new species appears to be constant across its range (verified for Isalo and Ankarafantsika). It is striking that the newly discovered candidate species *B.* sp. Ca12, has only been found at two sites, the small villages of Antsirasira and Maevatanana, both located in the lower Sambirano valley, whereas at two sites closer to the Sambirano river mouth, Benavony and Ambanja, we found *B. wittei*. Antsirasira is also the only known site from which the miniaturized species *Wakea madinika* is known (Vences *et al.* 2002), a deep mantellid lineage sister to the genus *Mantella* and superficially similar to *Blommersia*. This calls for further exploration of this area, which consists largely of anthropogenically transformed habitat covered with cocoa plantations, but may still harbor other undetected microendemic species.

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Supplementary Materials. The following supporting information can be downloaded at the DOI landing page of this paper.

SUPPLEMENTARY TABLE 1. GenBank accession numbers, voucher numbers and localities of all sequences used in the molecular analysis.

SUPPLEMENTARY TABLE 2. Measurements of all species of *Blommersia*.