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Communicator whistles: A Trek through the taxonomy of the *Boophis marojezensis* complex reveals seven new, morphologically cryptic treefrogs from Madagascar (Amphibia: Anura: Mantellidae)

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Abstract

The Malagasy stream-breeding treefrog species *Boophis marojezensis* contains bioacoustically and genetically highly divergent populations. Some of these populations have been defined as candidate species and emit somewhat bizarre advertisement calls consisting of multiple whistle-notes. We here enable a long-overdue taxonomic revision of this species complex by applying a museomics approach to sequence DNA from the holotype of *B. marojezensis.* Based on an integrative approach that combines divergence levels in mitochondrial DNA and in three nuclear-encoded genes, morphological data, and bioacoustic comparisons, we conclude that eight different species exist in this complex, seven of which are formally described herein as new. Although morphological differences between species are small and mainly separate small-sized from larger-sized species, conclusive evidence for the new species comes from their sympatric and sometimes syntopic occurrence without haplotype sharing in three nuclear genes and under maintenance of bioacoustic differences. Uncorrected genetic divergences in the mitochondrial 16S rRNA gene are >3% in almost all cases, and in some cases up to 8%. In reference to the otherworldly sounds by which these frogs fill Malagasy rainforests, some of them reminiscent of sounds of technical equipment in the fictional "Star Trek" universe, we here name and describe the seven new species in honor of fictional captains of starships, namely *B. kirki* **sp. nov.**, *B. picardi* **sp. nov.**, *B. siskoi* **sp. nov.**, *B. janewayae* **sp. nov.**, *B. archeri* **sp. nov.**, *B. pikei* **sp. nov.**, and *B. burnhamae* **sp. nov.** The majority of these species occur in northern Madagascar, where up to three species can occur in immediate geographical proximity, e.g., *B. marojezensis*, *B. burnhamae* **sp. nov.** and *B. pikei* **sp. nov.** at different elevations in the Marojejy Massif. South of 16°S latitude, only *B. janewayae* **sp. nov.**, *B. picardi* **sp. nov.**, and *B. kirki* **sp. nov.** are found, with the latter extending southwards to Ranomafana National Park. Our study confirms the existence of numerous morphologically cryptic and microendemic species among Madagascar's amphibians, some of which are known only from unprotected sites and require adequate conservation management.

Keywords

Bioacoustics, *Boophis archeri* **sp. nov.**, *Boophis burnhamae* **sp. nov.**, *Boophis janewayae* **sp. nov.**, *Boophis kirki* **sp. nov.**, *Boophis picardi* **sp. nov.**, *Boophis pikei* **sp. nov.**, *Boophis siskoi* **sp. nov.**, cryptic species, integrative taxonomy, molecular genetics, museomics, systematics

Introduction

With 80 currently described species (AmphibiaWeb 2023), *Boophis* Tschudi, 1838 is the most speciose genus in the Malagasy-Comoran-endemic anuran family Mantellidae (Glaw and Vences 2006, 2007; Hutter et al. 2022). *Boophis* are treefrogs of relatively generalized reproductive behavior, typically laying their eggs into the water of streams or ponds where their exotrophic tadpoles develop (Blommers-Schlösser 1979b; Blommers-Schlösser and Blanc 1991; Hutter et al. 2022). Most *Boophis* species are stream breeding (Vences et al. 2002), partly with tadpoles that are morphologically adapted to lotic habitats (Blommers-Schlösser 1979b; Raharivololoniaina et al. 2006; Randrianiaina et al. 2009a, 2009b, 2012; Rasolonjatovo Hiobiarilanto et al. 2010; Grosjean et al. 2011). Many *Boophis* species are very vocal, their males emit loud and distinct advertisement calls that have proven useful both for monitoring and taxonomic purposes (Vences et al. 2008; Hutter et al. 2022). While closely related species of *Boophis* are often morphologically highly cryptic, they typically differ strongly in advertisement call parameters, especially when occurring in syntopy (e.g., Glaw and Thiesmeier 1993; Andreone et al. 2005; Glaw et al. 2001, 2010, 2021; Glaw and Vences 2002; Vences and Glaw 2002; Vences et al. 2012).

Morphological and ecological characters, and especially molecular phylogenetic studies, have revealed several well-supported clades that are defined as species groups within *Boophis* (Blommers-Schlösser 1979b; Blommers-Schlösser and Blanc 1993; Glaw and Vences 2006; Hutter et al. 2018). Of these, the *Boophis blommersae* group currently contains three nominal species: *B. blommersae* Glaw & Vences, 1994, *B. marojezensis* Glaw & Vences, 1994, and *B. vittatus* Glaw, Vences, Andreone & Vallan, 2001, plus seven candidate species phylogenetically related to *B. marojezensis*, but genetically highly distinct (Hutter et al. 2018). *Boophis marojezensis* and these candidate species (*B.* sp. Ca25, *B.* sp. Ca26, *B.* sp. Ca51, *B.* sp. Ca52, *B.* sp. Ca53, *B.* sp. Ca55, *B.* sp. Ca68; named after the scheme of Vieites et al. 2009 as refined by Perl et al. 2014) will hereafter be called the "*B. marojezensis* complex", and are the subject of this study.

Boophis marojezensis was assigned by Glaw et al. (2001) to the *B. majori* species group. Hutter et al. (2018) demonstrated this group to be paraphyletic, and thus erected the *B. blommersae* species group for a clade containing species with specialized "suctorial" tadpoles adapted to fast-flowing streams (Randrianiaina et al. 2012). The three nominal species in the group are distinct bioacoustically, and in part also morphologically: *B. marojezensis* has an advertisement call consisting of melodious whistles while *B. blommersae* emits series of pulsed notes, and *B. vittatus* a long series of clicks (Glaw et al. 2001). Furthermore, *B. vittatus* differs from the other two species by the presence of dark dorsolateral bands (Glaw et al. 2001). Already in Glaw et al. (2001), it was suspected that the specimens subsumed under the name *B. marojezensis* may belong to more than one species, due to substantial differences in calls: in some populations, a series of 3–5 regular whistles were recorded, and in other populations males emit outlandish sounds at high-pitched frequencies up to 6250 Hz, consisting of 21 notes of increasing duration and strong frequency modulation (Glaw et al. 2001; Vences et al. 2006; Rosa et al. 2011).

In a subsequent analysis of larval morphology, Randrianiaina et al. (2012) provided evidence for the existence of multiple additional candidate species in the *B. marojezensis* complex, primarily based on divergent DNA barcodes of the tadpoles studied. Their "reverse taxonomy" approach revealed genetic distances in the mitochondrial 16S rRNA gene >3% between sets of tadpoles that in some cases occurred in sympatry and were also characterized by distinct differences in color pattern. These genetic differences were also confirmed by a mitochondrial and nuclear multi-gene analysis of Hutter et al. (2018). However, a taxonomic revision of this group remained out of reach because for several of the genetically divergent lineages, only larval specimens but no adults had been collected, and no reliable call recording and no genetic data were available from the *B. marojezensis* type material. Therefore, the attribution of this nomen to one of the several genetic lineages occurring at its type locality, the Marojejy Massif, remained in need of clarification.

Here, we assembled a comprehensive set of new genetic, bioacoustic and morphological data to revise the taxonomy of the *B. marojezensis* complex. Based on extensive new collections, we compare the morphology and advertisement calls of adults of all genetic lineages, map their distribution, and assess their evolutionary independence by comparing differentiation in one mitochondrial and three nuclear-encoded markers. Furthermore, we obtained a DNA sequence of the holotype of *B. marojezensis*, thus allowing us to unambiguously clarify the identity of this nomen. Our combined datasets provide strong evidence for the existence of seven new species in the complex which, due to their truly alien-sounding advertisement calls, are herein named after prominent characters in the fictional "Star Trek" universe. With this, we primarily aim to honor the focus on inspiring – even

if not always accurate – science and nature conservation prevalent in numerous Star Trek episodes (e.g., Andreadis 1999; Jenkins and Jenkins 1999; Noor 2018), and the spirit of discovery and scientific exploration that it fosters. It seems also fitting that finding these frogs sometimes requires considerable trekking; pursuing strange new calls, to seek out new frogs in new forests; boldly going where no herpetologist has gone before.

Materials and Methods

We aimed to analyze all voucher specimens, samples and call recordings of the *B. marojezensis* complex available to us. This included material from multiple field expeditions from 1994–2022, primarily obtained by collecting calling males with the aid of flashlights at night. Specimens were anesthetized by immersion in aqueous solutions of tricaine methanesulfonate (MS222) or chlorobutanol, and subsequently euthanized by an overdose of the same substances. Tissue samples for molecular analysis were taken from the euthanized specimens and stored separately in 1.5 ml vials filled with pure ethanol. Vouchers were then fixed in 95% ethanol or in 4% formaldehyde solution, preserved in 70% ethanol, and deposited in the Biodiversity Institute and Natural History Museum of the University of Kansas (**KU**); Zoologisches Forschungsmuseum Alexander Koenig, Bonn (**ZFMK**); Museo Regionale di Scienze Naturali, Torino (**MRSN**); Zoologische Staatssammlung München (**ZSM**); and the Université d'Antananarivo, Mention Zoologie et Biodiversité Animale (**UADBA**). **FGZC**, **FGMV** and **ZCMV** refer to field numbers of F. Glaw and M. Vences. **FAZC** refers to field numbers of F. Andreone. **CRH** and **MSZC** refer to field numbers of Carl R. Hutter, and Mark D. Scherz respectively. Geographic regions within Madagascar are named according to Boumans et al. (2007) and Brown et al. (2016).

Morphometric measurements of voucher specimens were taken by MV with a manual caliper and an accuracy of 0.1 mm, as follows: snout–vent length (**SVL**); maximum head width (**HW**); head length from tip of snout to posterior edge of mouth opening (**HL**); horizontal tympanum diameter (**TD**); horizontal eye diameter (**ED**); distance between anterior edge of eye and nostril (**END**); distance between nostril and tip of snout (**NSD**); distance between both nostrils (**NND**); forelimb length, from limb insertion to tip of longest finger (**FORL**); hand length, from the articulation of the carpus with the radioulna to the tip of the longest finger (**HAL**); hindlimb length, from the cloaca to the tip of the longest toe (**HIL**); foot length (**FOL**); foot length including tarsus (**FOTL**); and tibia length (**TIBL**). Webbing formula is reported according to Blommers-Schlösser (1979a) to ensure comparability with previous species descriptions of Malagasy frogs. Explory statistical analyses of morphometric differences were carried out by computing and comparing potentially diagnostic measurements and ratios between species; consistent differences were found only in body size, and these were tested using a non-parametric Kruskal-Wallis ANOVA in Statistica v. 7 (Statsoft Inc., Tulsa, USA).

We recorded vocalizations in the field using a tape recorder (Tensai RCR-3222) with an external microphone (Vivanco EM238), a digital recorder with built-in microphones (Edirol R-09), and digital recorders (Marantz PMD 661-MkII, Olympus LS-10 Linear PCM Recorder) with external shotgun microphones (Sennheiser ME66+K6 and MKH 8060). In the cases of digital recorders, sound files were recorded at 44.1 kHz sample rate and saved in uncompressed WAV format. Recordings were sampled or re-sampled at 22.05 kHz and 32-bit resolution and analyzed using the software Cool Edit Pro 2.0. We obtained frequency information through Fast Fourier Transformation (**FFT**; width 1024 points) at Hanning window function. Spectrograms were produced at Blackman window function with 256 bands resolution. In some cases, filtering was used to remove background sounds, applied only to frequencies outside the prevalent bandwidths of calls. Temporal measurements are summarized as range with mean ± standard deviation in parentheses. Terminology and methods in call analyses and their descriptions follow the recommendations of Köhler et al. (2017), using the note-centered terminological scheme. Representative cuts of the call recordings analyzed were deposited in the Zenodo repository (https://doi.org/10.5281/zenodo.10557964).

Genetic divergence between individuals and lineages was assessed using DNA fragments of one mitochondrial and three nuclear-encoded genes. DNA was extracted from tissue samples using a salt-extraction protocol (Bruford et al. 1992). As mitochondrial marker, we PCR-amplified and sequenced the 3'-terminal fragment of the 16S rRNA gene (16S) using primers 16SAL (5'–CGCCTGTTTATCAAAAACAT–3') and 16SBH-new (5'–CCTGGATTACTCCGGTCTGA–3'), modified from Palumbi et al. (1991), with cycling protocol 94°C (90s), [94°C (45s), 55°C (45s), 72°C (90s)] × 33, 72°C (300s).

As nuclear-encoded markers, we sequenced the following gene fragments: (i) a fragment of the recombination activating gene 1 (RAG-1), using primers Gephlut-RAG1-F1 (5'–ATGGAGAGCCAACCCCTATC–3') and Gephlut-RAG1-R1 (5'–KCCAGACTCGTTTCCTTCRC–3'), and the sequencing primer RAG1-Manti-Seq1 (5'–GCAAAGCCVTTTATTGAAACC–3'), with cycling protocol: 94°C (120s), [94°C (20s), 54°C (50s), 72°C (180s)] × 39, 72°C (600s) (Vences et al. 2021a); (ii) a fragment of sacsin (SACS), amplified with a nested PCR approach following 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: 94°C(240s), [94°C (45s), 45°C (40s), 72°C (120s)] × 45, 72°C (600s); and (iii) a fragment of the KIAA1239 gene, with external primers KIAA1239-F1 (5'–CARCCTTGGGTNTTYCA–3'), KIAA1239-R1 (5'–CMACAAAYTGGTCRTTR–3'), and

internal primers KIAA1239-NF1 (5'–GAGCCNGAYAT-HTTYTTYG–3') and KIAA1239-NR1 (5'–TTCACRA-ANCCMCCNG–3') (Shen et al. 2012), with nested PCR and cycling protocols as those used for SACS.

Furthermore, to assess the phylogeny within the *B. marojezensis* group, we expanded the multi-gene data set of Hutter et al. (2018) and assembled a concatenated alignment of five gene fragments: for mitochondrial DNA, two fragments of mitochondrial 16S, one fragment each of 12S rRNA (12S), cytochrome *b* (cyt *b*), Cytochrome Oxidase Subunit 1 (COI), and NADH dehydrogenase subunit 1 (ND1); for nuclear DNA, KIAA1239, RAG-1 and SACS). For several lineages, sequences of 12S, cyt *b* and COI were added to the data set using primers and cycling conditions as in Hutter et al. (2018).

PCR products were purified with Exonuclease I and Shrimp Alkaline Phosphatase digestion and sequenced by LGC Genomics (Berlin) on an automated capillary sequencer. Chromatograms were checked for base-calling errors and edited with CodonCode Aligner v 3.7.1 (Codon Code Corporation, Dedham, MA, USA) and newly determined sequences submitted to GenBank (accession numbers PP931010–PP931012, PP932411–PP932463, PP932468–PP932472, PP934894–PP935008, PQ278105).

The holotype of *B. marojezensis* (ZFMK 57401), deposited at the Zoologisches Forschungsmuseum Alexander Koenig in Bonn, Germany, was sampled in 2022 for a small piece of thigh muscle tissue after inflicting a small incision and reflecting the ventral skin of the thigh. Sampling was done with sterile scalpels and tweezers, and the sample was stored in a vial with pure ethanol that had been previously filled in a laboratory naive to molecular work on mantellids. Prior to DNA extraction, the sample was weighed and incubated in a Guanidine Thiocyanate (GuSCN) based extraction buffer solution at 37°C overnight. The next day, a total volume of 25 µl genomic DNA was extracted following the protocol of Rohland et al. (2004), following several consecutive steps as described in Straube et al. (2021). The yield of DNA was quantified based on 1 µl DNA extract using the Qubit dsDNA HS Assay Kit 0.2–100 ng/µl (Life Technologies, Carlsbad, California, US) according to the instructions of the manufacturer. Subsequently, a maximum of 13 ng DNA was used as input for single-stranded library preparation following the protocol of Gansauge et al. (2017). All lab work prior to qPCR was conducted in a dedicated DNA facility at the University of Potsdam, Germany, which meets all requirements to work with historical samples (see Fulton and Shapiro 2019) and extraction and library blanks were run alongside all samples to check for contamination. Final library concentrations and fragment length distributions were assessed using a 2200 TapeStation (Agilent Technologies) assay. Sequence data was obtained through shotgun-sequencing of approximately five million 75-bp single-end reads using an Illumina Nextseq 500/550 sequencing platform at the University of Potsdam, following the procedure described in Paijmans et al. (2017). The quality of the obtained reads was visualized twice using FastQC (https://www.bioinformatics.babraham.ac.uk), both before and after trimming of Illumina adapter sequences and discarding reads shorter than 30 bp with cutadapt v1.12 (Martin 2011).

We used local Blast (Blast+; Camacho et al. 2009) against a library of 16S sequences of all *B. marojezensis* genetic lineages plus a selection of additional mantellid genera (*Mantidactylus*, *Spinomantis*, *Blommersia*), and collected the matching reads in a fasta file. We then used CodonCode Aligner v 3.7.1 (CodonCode Corporation) to map the matching reads to 16S reference sequences of various *B. marojezensis* lineages as well as additional mantellid genera (option: "align to reference"), verified the resulting assemblies were congruent (thus, no reference bias was introduced), and used the most complete consensus sequence of 16S obtained as representing the holotype, with missing sections in-between contigs coded by the letter "N".

We aligned sequences for 16S and each of the nuclear-encoded loci (KIAA1239, RAG-1 and SACS) individually in MEGA7 (Kumar et al. 2016) with the Clustal alignment option. All alignments as well as a table with metadata of voucher specimens (including sequence accession numbers) are available from the Zenodo repository (https://doi.org/10.5281/zenodo.10557964).

The 16S alignment contained 147 sequences for a total alignment length of 533 nucleotides and only required single gaps to account for indels. The entire alignment was therefore used for phylogenetic analysis. We inferred a Maximum Likelihood (**ML**) tree in RAxML (Stamatakis 2014) using raxmlGUI v. 2.0 (Edler et al. 2020), under a Hasegawa-Kishino-Yano (HKY+G) determined as the model best-fitting the data under the Bayesian Information Criterion in a model testing analysis performed in MEGA7, and available in RAxML. Node support was tested with 500 full ("thorough") bootstrap replicates. Furthermore, phylogeny was also assessed via Bayesian Inference (**BI**) with MrBayes 3.2 (Ronquist et al. 2012) under the same substitution model, for 5 million generations, sampling every 1000th tree, and a burn-in of 25% after empirically verifying stationarity of parameters.

For an objective inference of primary species hypotheses, we used ASAP (Puillandre et al. 2021) as implemented in iTaxoTools (Vences et al. 2021a) on the 16S data set. Pairwise sequence distances between ASAP-inferred lineages were then calculated using the program TaxI2 (Vences et al. 2021a). For ASAP and TaxI2, we used a reduced and trimmed alignment of 469 bp containing 92 sequences without missing nucleotides (i.e., removing sequences with large numbers of missing data, and trimming the entire alignment to equal length of remaining sequences). We used the same reduced 16S dataset, complemented with a full-length 16S sequence of *Mantella baroni* (Kurabayashi et al. 2008), to determine molecular diagnostic sites for the inferred species in the *B. marojezensis* complex using MolD (Fedosov et al. 2022) (alignments available from the Zenodo repository, https://doi.org/10.5281/zenodo.10557964).

For the multi-gene data set, sequences were aligned with the MAFFT G-Ins-I algorithm (Katoh and Stanley 2013) and concatenated using Concatenator (Vences et

Table 1. Uncorrected pairwise genetic distances in % (mean, with minimum and maximum in parentheses) within and between lineages of the *Boophis marojezensis* complex, calculated from a 469 bp alignment of the mitochondrial 16S rRNA gene containing 92 sequences without missing data. Cells along the diagonal show intra-lineage variation.

	F	H	A	G	C	B	D	E
Lineage F *B. marojezensis*	0.3 (0.0–0.4)							
Lineage H *B. burnhamae* **sp. nov.**	4.9 (4.7–5.2)	0.3 (0.0–0.6)						
Lineage A *B. kirki* **sp. nov.**	7.6 (6.9–8.1)	6.9 (6.6–7.7)	0.5 (0.0–2.6)					
Lineage G *B. siskoi* **sp. nov.**	5.5 (5.4–5.6)	3.8 (3.2–4.9)	6.4 (6.2–6.6)	0.7 (0.0–1.5)				
Lineage C *B. pikei* **sp. nov.**	3.1 (3.0–3.2)	4.1 (3.6–4.5)	7.2 (6.4–7.7)	4.5 (4.1–4.9)	0.3 (0.0–0.6)			
Lineage B *B. janewayae* **sp. nov.**	5.6 (4.7–7.1)	5.3 (4.7–6.9)	6.9 (6.0–7.7)	6.0 (5.4–7.5)	4.9 (4.3–6.4)	1.6 (0.0–4.1)		
Lineage D *B. picardi* **sp. nov.**	3.0 (2.8–3.2)	4.3 (3.9–4.7)	7.9 (7.5–8.4)	5.6 (5.4–5.8)	3.6 (3.2–3.9)	6.0 (5.4–7.5)	0.2 (0.0–1.3)	
Lineage E *B. archeri* **sp. nov.**	3.7 (3.4–3.9)	3.4 (3.2–3.6)	6.9 (6.7–7.1)	3.7 (3.4–4.5)	3.9 (3.7–4.1)	4.5 (4.1–5.8)	4.7 (4.5–4.9)	0.0 (0.0–0.0)

al. 2021) and submitted to phylogenetic analysis. The alignment in Phylip and Nexus formats, as well as a table detailing metadata of the samples used for each lineage and each gene fragment, are available from the Zenodo repository along with tree files of all analyses (https://doi.org/10.5281/zenodo.10557964). For this data set, we performed several alternative analyses: partitioned and unpartitioned ML searches using IQ-Tree v.2.2.2.6 (Nguyen et al. 2015) after determining substitution models and (for the partitioned analysis) data partitions using ModelFinder (Kalyaanamoorthy et al. 2017) and assessing bootstrap support with 2000 full non-parametric bootstrap replicates; and BI analysis using MrBayes 3.2 (Ronquist et al. 2012) using a GTR+G model and the same run specifications as for the 16S data set. Furthermore, analyses were repeated for data sets trimmed to contain the mitochondrial gene fragments only.

The alignments of the three nuclear-encoded genes were analyzed independently to understand concordance (or absence thereof) in the differentiation of these three unlinked genetic markers. We used a genealogy visualization approach to graphically represent the relationship among alleles (haplotypes). Haplotypes were estimated with the PHASE algorithm (Stephens et al. 2001), and a haplotype network using the TCS algorithm (Templeton et al. 1992) was constructed in Hapsolutely (part of iTaxoTools).

We follow the general lineage concept (de Queiroz 1998, 2007) in combination with a relaxed biological species criterion, i.e., demanding indications for reproductive isolation, i.e., indications for restricted gene flow among lineages (e.g., criteria used for the species list of the European herpetofauna: Speybroeck et al. 2020). Because reproductive barriers generated through time increase genealogical depth and agreement among unlinked loci (Avise and Wollenberg 1997), we use genealogical concordance (Avise and Ball 1990) between mitochondrial and nuclear loci, especially in populations occurring in sympatry or close geographical proximity, as an approximative indicator for restricted gene flow. Species status is then assigned to lineages based on combined evaluation of genetic, morphological and bioacoustic evidence (Padial et al. 2010).

Results

Molecular differentiation

The phylogenetic analysis of the full 16S alignment revealed 8 major clades with bootstrap support of 65–99% which we here name as mitochondrial lineages A–H (Fig. 1). ML and BI recovered largely congruent trees, with minimal differences in the poorly supported nodes. Of these, clade A was divided into highly supported subclades here named A1–A2, and clade B also included substantial sequence variation up to 4.1% uncorrected pairwise distances (Table 1) which however was not resolved into strongly supported subclades. Relationships among the main lineages were in general poorly resolved, but a clade containing lineage C, D and F received 80% bootstrap support, and the clade containing lineages D and F received 73% bootstrap support.

The analysis with ASAP suggested with the best (lowest) ASAP score of 2.0 is a species partition with 10 subsets (= primary species hypotheses). These corresponded exactly to lineages A–H revealed by the phylogenetic analysis, except for separating lineage A into A1 and A2, and placing one specimen of lineage B (from Makira) into a separate subset. To take a conservative approach, we followed the fourth best ASAP partition (score 4.5) which recovered all of lineage B specimens as a single subset, but still separated A1 and A2 as different subsets.

Sequence divergences between the main mitochondrial lineages were high, with average uncorrected pairwise distances in all cases amounting to 3.0% or more (Table 1), and only the minimum distance between lineage D

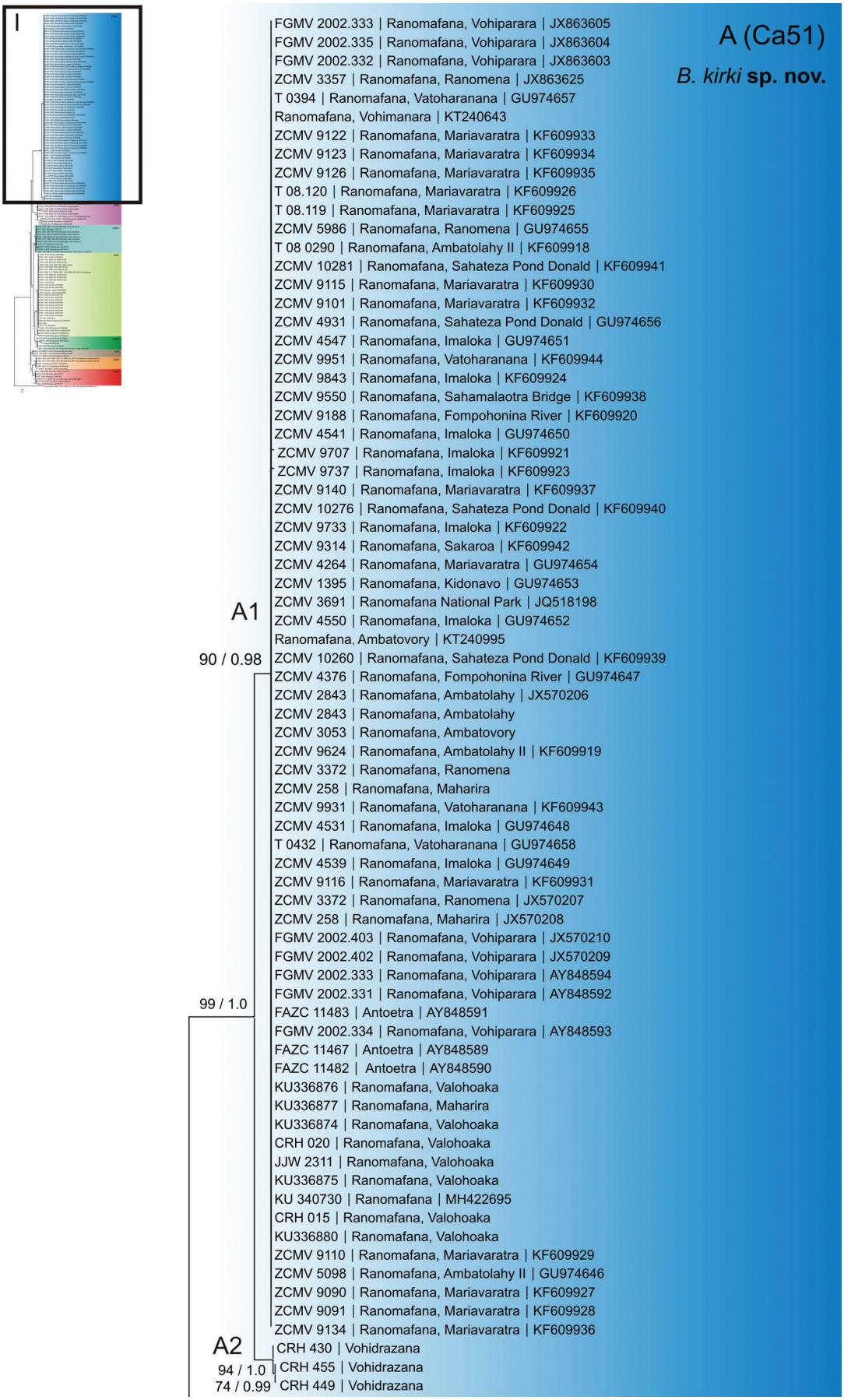

Figure 1 (Part A). Maximum Likelihood tree inferred from an alignment of 533 nucleotides of the mitochondrial 16S rRNA gene for 146 samples of the *Boophis marojezensis* complex and one outgroup (*B. picturatus*). Main lineages A–H are as discussed in the text and candidate species numbers are assigned to lineages based on previous studies. Numbers at nodes are bootstrap proportions (in percent) and Bayesian posterior probabilities (not shown for some of the most shallow nodes).

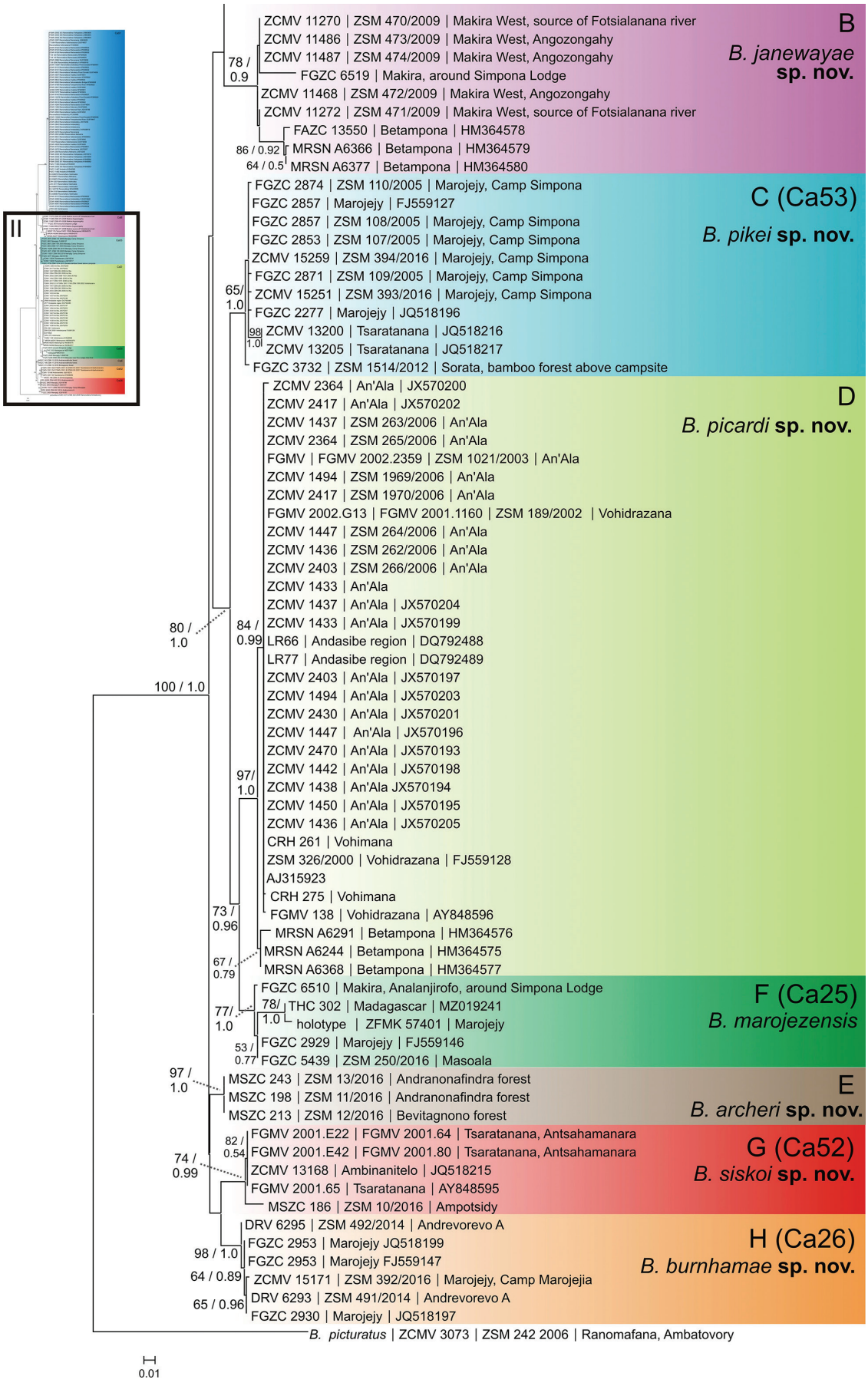

Figure 1 (Part B). Maximum Likelihood tree inferred from an alignment of 533 nucleotides of the mitochondrial 16S rRNA gene for 146 samples of the *Boophis marojezensis* complex and one outgroup (*B. picturatus*). Main lineages A–H are as discussed in the text and candidate species numbers are assigned to lineages based on previous studies. Numbers at nodes are bootstrap proportions (in percent) and Bayesian posterior probabilities (not shown for some of the most shallow nodes).

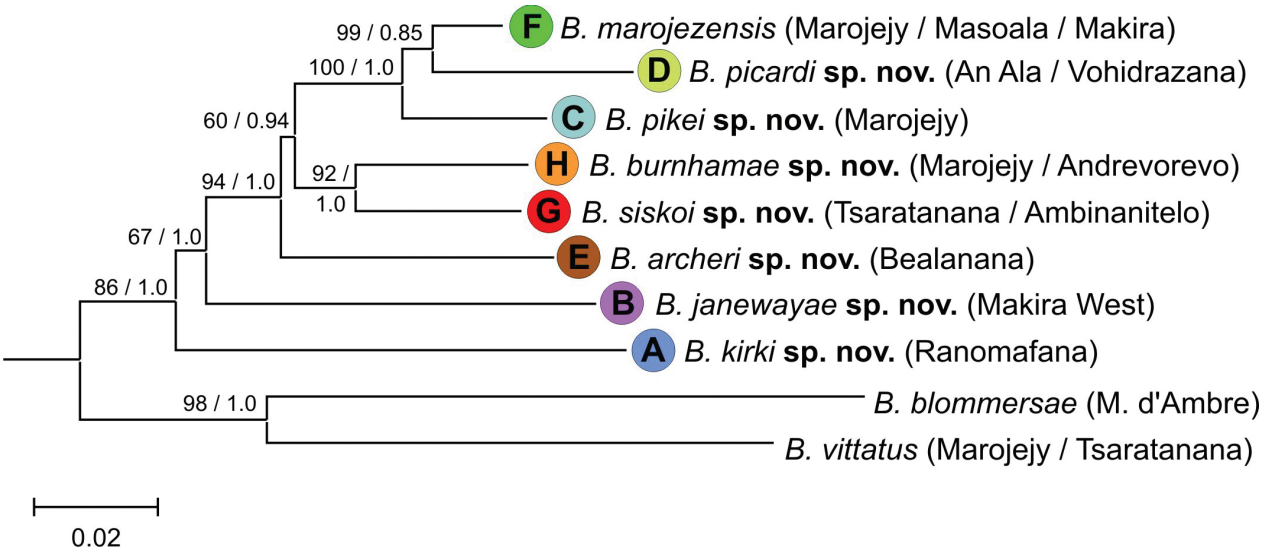

Figure 2. Maximum Likelihood tree inferred from an alignment of 6741 nucleotides of the mitochondrial 12S rRNA and 16S rRNA, ND1, COI and cyt *b* genes, and the nuclear KIAA1239, RAG-1 and SACS genes for the main lineages of the *Boophis marojezensis* group. The tree was rooted with *B. picturatus* (graphically removed to better illustrate branch lengths within the *B. marojezensis* group. Main lineages A–H of the *B. marojezensis* complex are as discussed in the text and in Figure 1. Values at nodes are bootstrap support in percent from a partitioned ML analysis with IQ-Tree, and posterior probabilities from an unpartitioned Bayesian Inference analysis with MrBayes.

and F being slightly below this value (2.8%). In some cases, very high distances were recorded, amounting to maxima of up to 8.1% (A vs. F), 7.7% (A vs. H and A vs. B), and 7.5% (B vs. G); this strongly suggests that the complex consists of more than one species, given that such high 16S distances have never been recorded within a single Malagasy anuran species. Distances within the main lineages were <2% with two exceptions: up to 2.6% in lineage A (marking the difference between A1 and A2), and up to 4.1% in lineage B, marking differences between several geographically disparate populations which will be further discussed below.

The 16S sequence assembled from Illumina reads of shotgun sequencing of DNA from the holotype of *B. marojezensis* (ZFMK 57401) contained a stretch of 191 nucleotides that covered the most variable and thus most phylogenetically informative part of the gene fragment. The sequence was placed with high bootstrap support (77%) in clade F, suggesting this clade corresponds to *B. marojezensis* sensu stricto, and not lineage C, which has previously been considered *B. marojezensis* sensu stricto in DNA barcoding schemes following Vieites et al. (2009; see Identity section of *B. marojezensis* in taxonomic accounts below).

The multi-gene phylogenetic analysis inferred using different methods and data sets (mitochondrial-only vs. mitochondrial+nuclear gene fragments; ML vs. BI, partitioned vs. unpartitioned) all recovered topologies almost identical to the one shown in Figure 2 for partitioned ML analysis; the only difference concerned the most weakly supported node where the partitioned ML analysis for mitochondrial gene fragments only had lineage E sister to the G+H clade rather than to the C+D+F+G+H clade. The analyses confirmed monophyly of the *B. marojezensis* complex (bootstrap support BS = 86% / Bayesian Posterior Probability PP = 1.0), which was sister to a clade containing *B. blommersae* and *B. vittatus*. Within the *B. marojezensis* complex, lineage A was sister to a clade containing all other lineages (BS = 67% / PP = 1.0), and lineage B split from the next node being sister to a clade of the remaining species (BS = 94% / PP = 1.0). Two further subclades were consistently highly supported: one containing lineages G and H (BS = 92% / PP = 1.0), and one containing lineages C, D and F (BS = 100% / PP = 1.0). Within the latter, the clade of D and F was supported by BS (99%) but not PP (0.85) values.

The analysis of haplotype genealogies for the three nuclear gene fragments (RAG-1, SACS, KIAA1239: alignment length 474, 960, and 726 nt; Fig. 3) gave mixed results but supported at least in some cases a lack or rarity of allele sharing between individuals belonging to different ASAP-defined mitochondrial lineages. Importantly, in all three networks, specimens of lineage A were placed in separate phylogroups without sharing any allele with other lineages. Similarly, lineage D had a limited amount of haplotype sharing, forming a totally independent phylogroup in RAG-1 and sharing alleles only with lineage F in SACS. Furthermore, exclusive alleles characterized lineage H in KIAA1239 and lineage B in SACS. Haplotype sharing in all three genes was most pervasive in alleles placed centrally in the networks, where for example in KIAA1239 the central allele was found in four different lineages, and in RAG-1 where the central allele was found in three different lineages, suggesting that this pattern most likely reflects incomplete lineage sorting rather than recent gene flow. A more detailed look at some of the allele sharing patterns revealed that in some cases alleles are shared between lineages that differ strongly in advertisement calls (see section on bioacoustics below), for instance lineages C and E, which share one allele in SACS, and lineages C

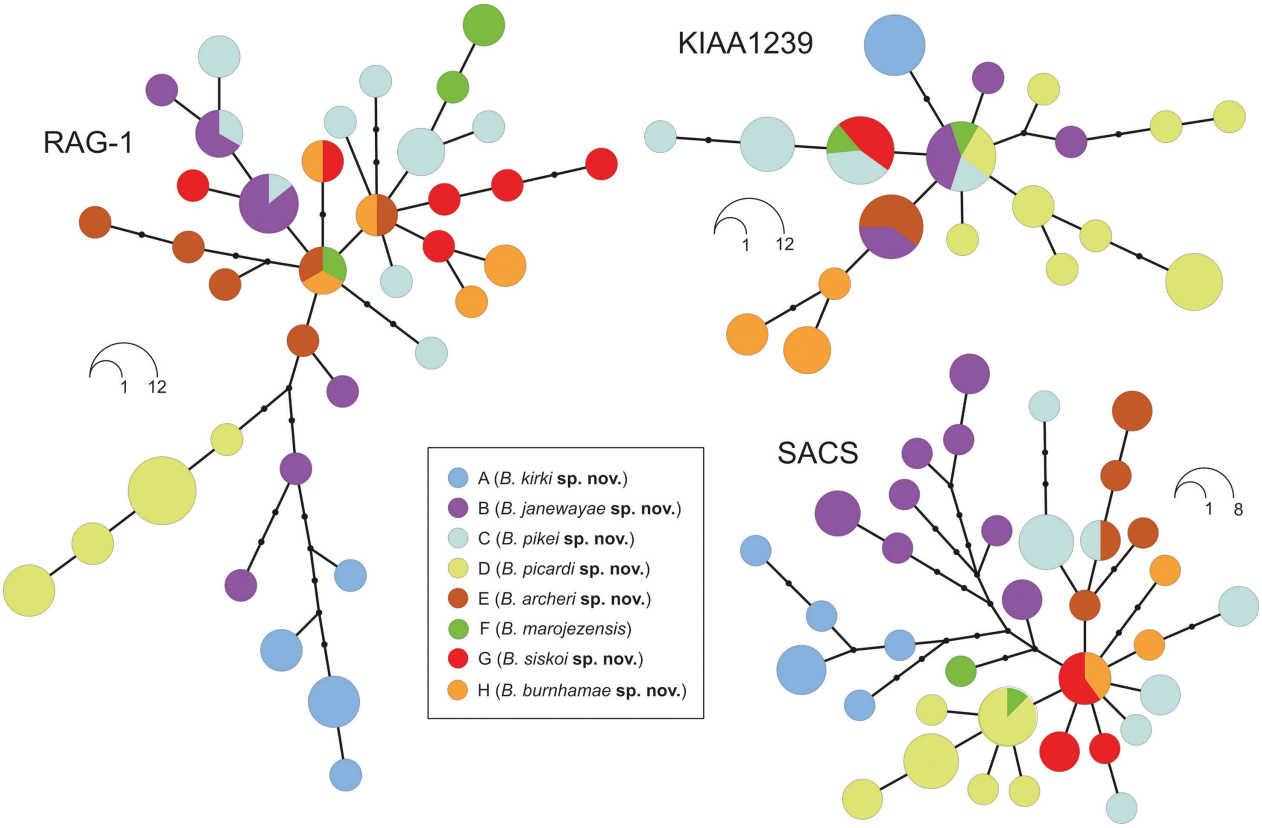

Figure 3. Haplotype genealogies for three fragments of nuclear-encoded single-copy genes, RAG-1 (474 nucleotides; 37 samples), SACS (960 nt; 36 samples) and KIAA1239 (726 nt; 37 samples). Sequences were phased previous to the analysis, and each sample is therefore represented by two sequences in each network. Coloring of samples is according to their assignment to main mitochondrial lineages in Figure 1. Circles represent haplotypes, with size proportional to allele frequency. Dots on the branches indicate the number of mutational steps between haplotypes.

and B, which share two alleles in RAG-1. The three lineages C, F and H that co-occur on the Marojejy Massif (at different elevations) show only extremely limited allele sharing: lineage F and H share the central allele in RAG-1, and F and C share two alleles in KIAA1239.

Geographical distribution of lineages

With reference to geographic regions as defined by Boumans et al. (2007) and Brown et al. (2016), the center of richness of mitochondrial lineages of the *B. marojezensis* complex is in northern Madagascar (Fig. 4). Lineages C, E, G and H have exclusively been recorded in the three northern regions (Sambirano, North, and North East), while lineage F inhabits the North East but also just reaches the Northern Central East in Makira. Lineage B and D occur in the Northern Central East while lineage A inhabits the Southern Central East and Northern Central East. Sympatry was observed at several sites: In the Marojejy Massif (lineages C, F and H), Betampona (lineages B and D), Vohidrazana (lineages A and D), and Makira (lineages B and F). While some lineages have rather wide elevational ranges (e.g., lineage H, 616–1717 m above sea level), others seem to be specialized to higher elevations (e.g., lineage A, 950–1420 m a.s.l.; lineage C, 1325–1699 m a.s.l.; lineage E, 1016–1169 m a.s.l.; lineage G, 1000–1320 m a.s.l.) or lower elevations (lineage F, 21–410 m a.s.l.), respectively (for details, see species accounts below).

Morphology

With a few exceptions (especially, topotypical individuals of lineage D) our morphological comparisons are exclusively based on measurements and color patterns of genotyped specimens included in the mitochondrial tree (Fig. 1).

Compared to several other small-sized and predominantly brownish species of *Boophis* such as *B. majori*, *B. miniatus*, or *B. picturatus*, most lineages of the *B. marojezensis* complex (and also of the wider *B. blommersae* group, i.e., including *B. blommersae* and *B. vittatus*) can be recognized by the lack of deep red or purple color ventrally on hindlimbs or feet (see also Glaw et al. 2001; Glaw and Vences 2007). In some specimens, especially of lineage A and D, such red or orange color is however visible, although it appears to fade in preservative as none of the preserved voucher specimens examined have such red color visible.

A clear distinction from all other brown-colored *Boophis* of other species groups is observed in tadpole morphology. As summarized by Randrianiaina et al. (2012), all known *B. blommersae* group tadpoles are character-

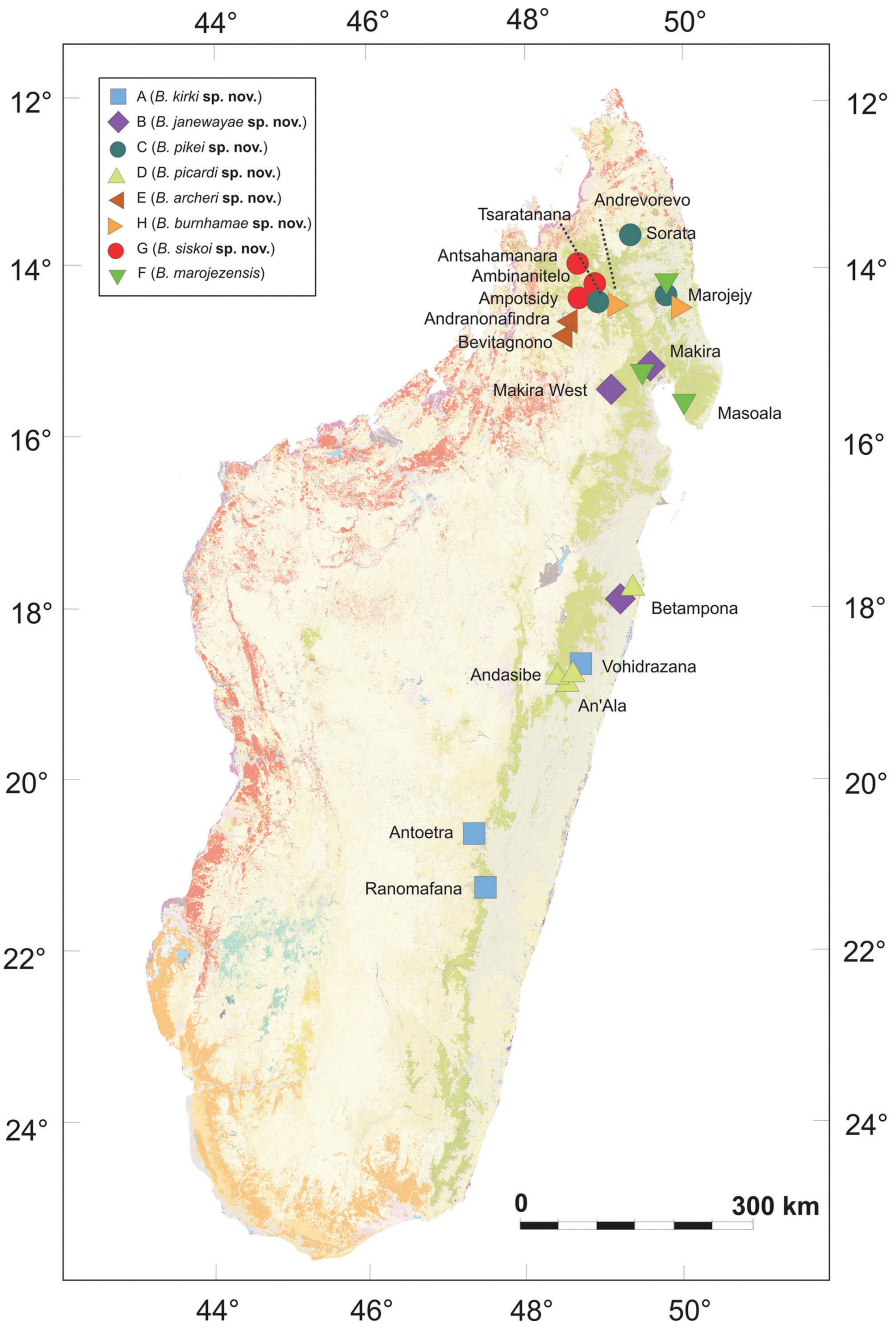

Figure 4. Map of Madagascar showing the distribution of species of the *Boophis marojezensis* complex. Only sites confirmed by molecular data are shown. Basemap shows vegetation across Madagascar from the Madagascar Vegetation Mapping Project (Moat and Smith 2007; formerly available at www.vegmad.org). Vegetation is colored as follows: green, humid forest (rainforest); red, western dry deciduous forest; bluish, western subhumid forest; orange, south western dry spiny forest-thicket; yellow, tapia forest.

ized by a large oral disc with many papillae arranged without dorsal gap, typical for the "suctorial" guild that is not found in any other *Boophis*.

However, within the *B. marojezensis* complex reliably assigning individuals to species based on morphology alone seems to be impossible. Some lineages appear to slightly differ in the amount of webbing, but differences are rather faint and it is uncertain whether they reflect individual, intra-lineage variation. Similarly, dorsal color can be quite variable within lineages, with pink markings present in some individuals of lineages A, C, D and F, and we suspect this color pattern may occasionally occur also in the other lineages. We identified two characters that, despite intra-lineage variation, might represent genuine differences between lineages. First, body size appears to be overall smaller in some lineages than in others; using measurements of males in Table 2 suggests highly significant differences between species (Kruskal-Wallis ANOVA, P=0.001). Of the lineages with sufficient specimens, it appears that lineages C and D have overall rather small body sizes (male SVL 21.4–25.0 and 21.3–23.2 mm, respectively) whereas lineages B and G are distinctly larger, almost without overlap (male SVL 25.2–28.8 and 25.0–27.2 mm, respectively). Secondly, in lineages A and D, specimens with distinctly orangish to red outer iris area occur which have not been observed in any other lineage, but this is not a fully diagnostic character as some individuals of both A and D lack this pattern. Randrianiaina et al. (2012) furthermore reported on differences in tadpole morphology: the larvae of lineage C (referred to as *B. marojezensis* and Ca53 in Randrianiaina et al. 2012) and lineage H (Ca26) have a clear, non-pigmented lateral area surrounding the body which is lacking in lineage A (Ca51) and G (Ca52), and the larvae of lineage F (Ca25 =

Table 2. Morphometric measurements (all in mm) of voucher specimens of the *Boophis marojezensis* complex. See Materials and Methods for abbreviations of measurements. M, male (possible young or subadults in parentheses); F, female, HT; holotype, PT; paratype; NS, not sequenced; NM, not measured. Measurements marked with asterisks are taken from Glaw et al. (2001).

Species/ mt lineage	Catalogue number	Field number	Locality	Status	Sex	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIBL
B. archeri **sp. nov.**																			
E	ZSM 12/2016	MSZC 213	Bealanana, Bevitagnono Forest	HT	M	25.3	9.7	9.3	[1.7]	4.0	2.2	2.6	3.0	15.3	7.8	41.3	17.3	10.3	13.0
E	ZSM 11/2016	MSZC 198	Bealanana, Andranonafindra Forest	PT	M	25.0	9.7	9.4	1.7	4.1	2.3	2.4	2.7	15.3	8.0	41.0	17.5	10.5	13.0
E	ZSM 13/2016	MSZC 243	Bealanana, Andranonafindra Forest	PT	F	31.1	11.4	11.2	2.0	4.4	2.8	2.7	3.3	19.6	9.8	51.6	22.9	13.5	16.0
B. burnhamae **sp. nov.**																			
H	ZSM 492/2014	DRV 6295	Andrevorevo A	HT	M	25.5	10.2	9.8	1.7	4.0	2.1	2.0	3.3	15.9	8.3	43.7	18.3	11.0	13.6
H	ZSM 392/2016	ZCMV 15171	Marojejy, Camp Marojejia	PT	(M)	20.4	7.8	7.5	NM	3.9	1.7	1.9	2.6	12.9	6.5	36.1	14.5	8.1	10.7
H	ZSM 491/2014	DRV 6293	Andrevorevo A	PT	F	31.9	11.6	12.7	2.1	4.3	3.2	2.7	3.3	21.8	9.8	54.4	22.6	13.0	17.1
B. kirki **sp. nov.**																			
NS	ZFMK 62300		Ranomafana		M	23.0	8.3	8.5	3.2	1.3	1.3	1.5	2.1	16.2	7.6	39.2	17.1	9.9	NM
NS	MRSN A658		Vohiparara		M	22.0	8.1	8.2	3.1	1.6	1.7	1.6	2.0	14.7	7.5	40.0	16.9	9.8	NM
NS	MRSN A1650		Vohiparara		M	20.0	7.3	7.6	3.1	1.6	1.7	1.5	2.2	13.4	6.6	37.1	15.5	9.0	NM
A	ZSM 699/2003	FGMV 2002.331	Ranomafana, Vohiparara	HT	M	23.4	8.9	8.0	1.8	3.6	2.0	1.5	2.2	15.5	7.1	41.5	17.6	10.5	13.0
A	ZSM 700/2003	FGMV 2002.332	Ranomafana, Vohiparara	PT	M	23.4	8.9	8.7	1.5	3.6	2.0	1.6	2.3	15.0	7.6	37.0	16.8	9.8	11.8
B. marojezensis																			
F	ZSM 250/2016	FGZC 5439	Masoala, near Eco-Lodge "Chez Arol"		M	24.7	9.4	9.2	[1.8]	3.9	2.4	1.9	2.7	14.5	6.9	39.0	17.1	10.5	12.3
F	ZSM 208/2022	FGZC 6510	Makira East, near Simpona Lodge		(M)	20.0	8.3	8.0	NM	3.4	2.1	1.7	2.0	14.9	6.5	35.4	15.5	9.1	11.6
F *	ZFMK 57401		Marojejy	HT	M	25.7	9.7	9.8	3.4	1.3	2.0	2.2	2.7	15.0	7.6	43.2	17.9	10.3	NM
NS	ZSM 567/1999 (ex ZFMK 57402)		Marojejy	PT	M	24.9	9.5	9.2	3.8	1.4	2.0	2.0	3.0	14.4	7.4	43.8	18.1	10.4	NM

Species/ mt lineage	Catalogue number	Field number	Locality	Status	Sex	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIBL
B. picardi **sp. nov.**																			
NS *	ZFMK 60085		An'Ala		M	21.3	7.9	7.9	2.7	1.2	1.6	1.6	2.2	15.2	7.2	39.6	16.2	9.3	NM
NS *	ZFMK 60086		An'Ala		M	21.3	8.1	8.9	3.1	1.1	1.8	1.7	2.2	14.0	6.9	39.3	16.0	9.2	NM
NS *	ZFMK 60087		An'Ala		M	21.9	8.0	8.0	3.1	1.2	1.8	1.9	2.1	14.7	6.8	39.7	16.0	8.8	NM
NS *	ZFMK 60088		An'Ala		M	22.5	7.9	8.2	3.3	1.2	1.7	1.6	2.1	14.2	6.8	38.3	16.2	9.6	NM
NS *	ZFMK 60089		An'Ala		M	21.5	7.6	7.6	3.0	0.9	1.6	1.6	1.9	15.0	6.5	38.6	15.6	8.9	NM
D	ZSM 326/2000		Vohidrazana	PT	M	21.9	8.4	8.6	NM	3.8	2.0	1.6	2.5	14.4	6.9	38.6	16.4	9.3	12.4
D	ZSM 330/2000		Vohidrazana	PT	M	21.8	8.6	8.7	1.6	3.8	2.0	1.9	2.6	14.7	7.0	37.9	16.3	9.5	11.8
D	ZSM 189/2002	FGMV 2001.1160	Vohidrazana	PT	M	23.2	8.8	8.2	1.7	4.0	2.0	1.8	2.6	15.0	7.4	39.7	16.8	10.7	12.4
D	ZSM 262/2006	ZCMV 1436	An'Ala	PT	M	21.3	8.1	8.3	1.7	3.7	2.0	1.9	2.4	14.2	6.7	35.4	15.6	8.4	11.1
D	ZSM 263/2006	ZCMV 1437	An'Ala	PT	M	21.5	8.0	8.2	1.8	4.1	1.6	2.0	2.8	14.2	6.6	36.3	15.2	7.8	11.6
D	ZSM 264/2006	ZCMV 1447	An'Ala	HT	M	23.0	8.6	8.1	NM	4.0	2.0	1.6	2.8	15.0	7.6	39.3	16.7	9.7	12.6
D	ZSM 265/2006	ZCMV 2364	An'Ala	PT	M	21.8	7.7	8.3	1.5	3.4	1.6	1.5	2.7	13.8	7.0	37.0	15.1	8.4	11.8
D	ZSM 266/2006	ZCMV 2403	An'Ala	PT	M	23.0	8.6	8.0	1.7	3.9	1.8	2.0	2.9	14.0	7.4	40.4	17.0	9.7	12.5
D	ZSM 1969/2006	ZCMV 1494	An'Ala	PT	M	22.8	8.4	8.3	1.7	3.4	1.9	1.7	2.6	14.8	7.2	39.4	16.1	9.7	12.8
D	ZSM 1970/2006	ZCMV 2417	An'Ala	PT	M	23.0	8.0	8.2	1.7	3.5	2.2	1.4	2.3	14.1	7.0	37.9	15.8	9.4	12.2
B. pikei **sp. nov.**																			
C	ZSM 393/2016	ZCMV 15251	Marojejy, Camp Simpona	HT	M	22.3	8.6	8.5	1.7	3.4	2.0	2.0	3.0	15.0	7.7	39.1	16.8	9.5	12.3
C	ZSM 394/2016	ZCMV 15259	Marojejy, Camp Simpona	PT	M	21.9	8.5	8.0	1.2	3.5	1.7	2.0	2.9	15.1	7.0	38.4	15.5	9.5	12.1
C	ZSM 1515/2012	FGZC 3657	Sorata	PT	M	23.1	8.9	8.7	1.6	3.5	1.6	1.7	2.8	15.8	7.8	41.4	17.2	10.3	12.8
C	ZSM 1514/2012	FGZC 3732	Sorata	PT	M	25.0	9.2	9.2	1.5	3.7	1.8	1.7	2.8	17.5	8.5	41.6	18.2	11.2	12.7
C	ZSM 108/2005	FGZC 2857	Marojejy, Camp Simpona	PT	M	21.4	8.3	9.2	1.8	4.0	1.9	2.0	2.6	14.0	7.3	39.4	16.3	10.3	12.0
C	ZSM 109/2005	FGZC 2871	Marojejy, Camp Simpona	PT	M	21.5	8.4	8.5	1.7	3.8	1.8	1.9	2.9	13.5	7.4	36.5	14.7	9.1	11.3
C	ZSM 110/2005	FGZC 2874	Marojejy, Camp Simpona	PT	M	21.5	8.0	7.8	1.8	3.6	1.6	1.8	2.5	14.0	7.0	37.2	15.5	8.8	11.5
B. siskoi **sp. nov.**																			
G	ZSM 10/2016	MSZC 186	Ampotsidy	PT	M	25.5	10.2	10.0	[1.7]	4.2	2.9	2.3	3.0	15.3	8.4	42.6	19.0	11.2	13.1
G	ZSM 614/2001	FGMV 2001.64	Tsaratanana, Antsahamanara	HT	M	25.8	10.0	9.5	1.8	4.6	2.4	2.0	2.6	16.8	7.8	45.7	18.5	11.0	14.1
G	ZSM 615/2001	FGMV 2001.71	Tsaratanana, Antsahamanara	PT	M	25.0	9.7	9.1	1.8	3.9	1.9	2.0	2.8	15.9	8.5	44.7	19.2	11.1	13.5
G	ZSM 616/2001	FGMV 2001.80	Tsaratanana, Antsahamanara	PT	M	27.2	10.2	10.0	1.9	4.0	2.4	2.2	3.0	16.7	8.8	45.5	20.0	11.3	14.5

Species/ mt lineage	Catalogue number	Field number	Locality	Status	Sex	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIBL
B. janewayae **sp. nov.**																			
B	ZSM 471/2009	ZCMV 11272	Makira West, Fotsialanana source	PT	M	27.7	10.3	10.2	2.0	4.6	2.3	2.0	3.5	18.0	9.5	47.1	20.5	13.0	14.1
B	ZSM 470/2009	ZCMV 11270	Makira West, Fotsialanana source	PT	M	28.8	10.9	10.6	1.9	4.5	2.7	2.2	3.3	18.4	9.4	47.2	21.2	13.3	14.6
B	ZSM 472/2009	ZCMV 11468	Makira West, Angozongahy	HT	M	27.4	10.2	9.8	2.0	3.7	2.1	2.0	2.9	17.8	9.2	46.9	21.2	13.7	14.4
B	ZSM 473/2009	ZCMV 11486	Makira West, Angozongahy	PT	M	27.2	10.3	10.3	1.6	4.0	2.2	2.1	2.7	17.2	8.8	47.6	20.6	13.8	14.7
B	ZSM 474/2009	ZCMV 11487	Makira West, Angozongahy	PT	M	26.9	9.9	9.7	1.9	4.0	1.8	2.3	3.0	17.0	8.4	42.0	19.0	11.0	13.4
B	ZSM 207/2022	FGZC 6519	Makira, East near Simpona Lodge	PT	M	25.2	9.5	9.7	NM	4.0	2.7	1.9	2.9	16.6	8.1	44.5	18.2	11.2	13.9

true *B. marojezensis*) have clear, rounded patches on the tail muscle.

Bioacoustics

Available recordings of vocalization of the *Boophis marojezensis* complex all contain calls composed of multiple tonal notes that are repeated in a regular pattern. These multi-note calls are herein considered to represent regular advertisement calls. Among the calls analyzed, some are simple in structure, whereas in other cases they are more complex, being composed of two different note types.

Two likely sister lineages, the nominal *Boophis marojezensis* and lineage D (Fig. 2), emit advertisement calls that are composed of two different note types, namely very short initial notes followed by notes of substantially longer duration. However, the calls of *B. marojezensis* differ at least from those of lineage D by a distinctly lower number of notes per call (7–8 vs. 17–25 notes/call). Calls of all remaining lineages exhibit only one type of note, although these notes may vary in temporal or spectral characters within the call. Calls of lineage B are distinguished from all other known calls in this complex by very long note duration (238–604 vs. 12–308 ms in other lineages) and longest inter-note interval duration (141–639 ms). Furthermore, they show the lowest values in dominant frequency (2687–3404 vs. 3499–5819 Hz in the other lineages), which is likely explained by their larger relative body size. Lineage B and lineage E share a low number of notes per call (3–5 and 3–6, respectively), but apart from shorter note duration, lineage E also differs by shorter call duration (889–1582 vs. 1571–2846 ms). The highest number of notes per call is evident in calls of lineage C (25–33 vs. 3–19 notes/call in all other lineages with only one note type). Calls of the distantly related lineages A and G share many parameters, but calls of lineage G tend to have a slight downward frequency modulation across the call, whereas in lineage A calls exhibit a slight upward frequency modulation. Unfortunately, call recordings for lineage H are lacking.

In summary, bioacoustic differences of advertisement calls in this species complex, quantitative and/or qualitative (see Fig. 5; Table 3), are in support of the phylogenetic lineages identified. The call differences observed are beyond those usually considered to represent intraspecific variation (see Köhler et al. 2017), allow for bioacoustic lineage identification and thus provide additional evidence for evolutionary lineage divergence and thus species status of these. This is particularly true for cases of syntopy such as in the lineages A and D at Vohidrazana in the Andasibe region.

Taxonomic conclusion

Taken together, the data we have amassed in this study provide overwhelming evidence for the existence of more than one species in the *B. marojezensis* complex. The eight main ASAP-delimited mitochondrial lineages iden-

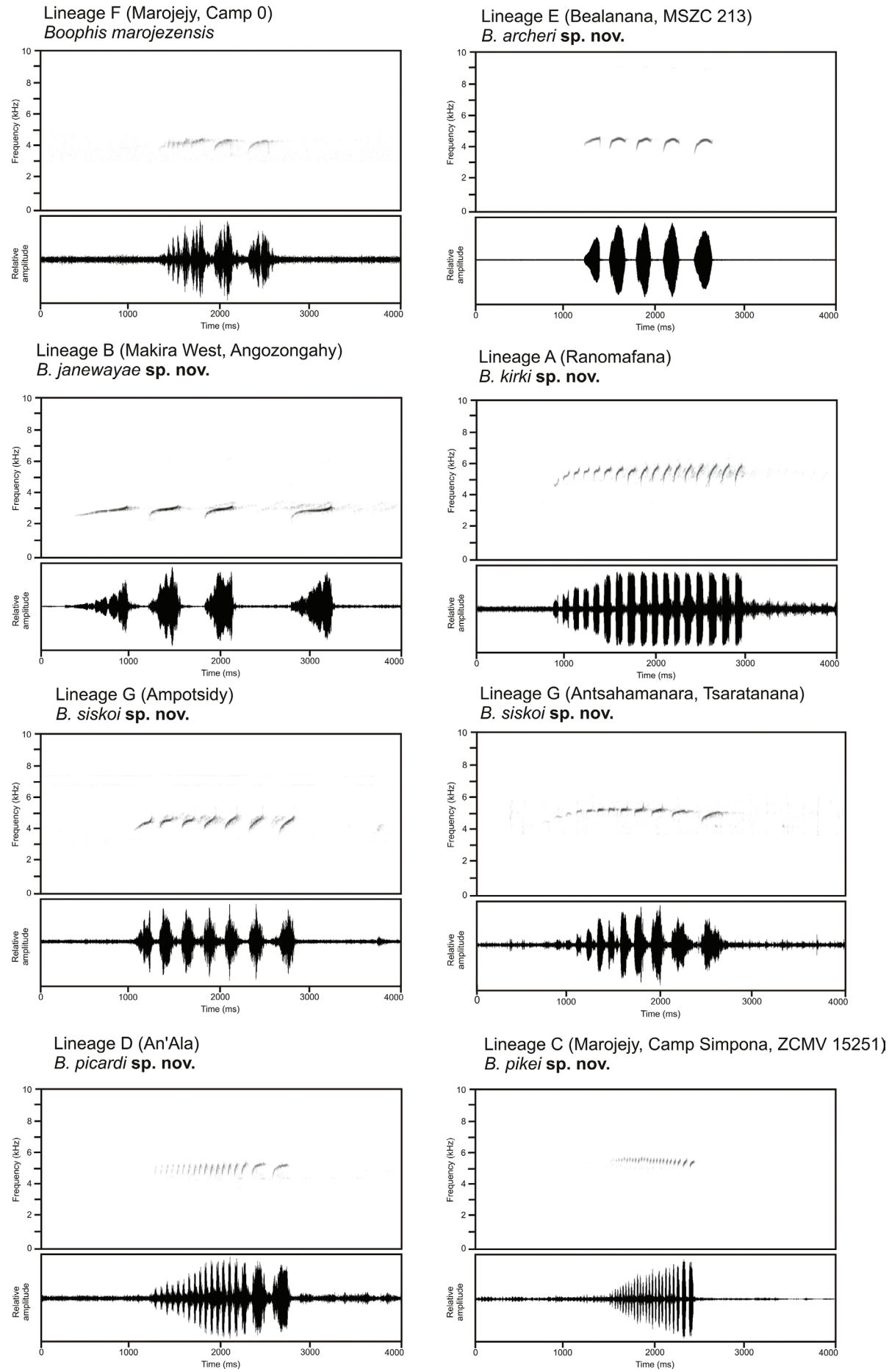

Figure 5. Comparative spectrograms and oscillograms illustrating representative calls of various species in the *Boophis marojezensis* complex, all at 4000 ms time scale.

Table 3. Summary and comparison of numerical advertisement call parameters among the major lineages (species) in the *Boophis marojezensis* complex.

	call duration [ms]	notes/call	note duration [ms]	inter-note intervals [ms]	dominant frequency [Hz]
Lineage A (*B. kirki* **sp. nov.**)	1180–2630	9–19	54–105	33–104	3499–5604
Lineage B (*B. janewayae* **sp. nov.**)	1571–2846	3–5	238–604	141–639	2687–3404
Lineage C (*B. pikei* **sp. nov.**)	921–1218	25–33	12–98	17–31	5174–5507
Lineage D (*B. picardi* **sp. nov.**)	1554–2388	17–25	19–78 (short) 90–225 (long)	25–145	4903–5819
Lineage E (*B. archeri* **sp. nov.**)	889–1582	3–6	158–308	77–162	4130–4799
Lineage F (*B. marojezensis*)	764–1222	7–8	15–51 (short) 142–259 (long)	19–179	4118–4441
Lineage G (*B. siskoi* **sp. nov.**)	1549–2168	7–12	44–220	41–180	4688–5332

tified differ by average distances >3% and thus above the threshold defined by Fouquet et al. (2007) and Vieites et al. (2009) to define candidate species. Several lineages are characterized by exclusive alleles and phylogroups in nuclear genes, a pattern most consistently applying to lineage A. Morphologically, several lineages differ without overlap in body size among males, lineages A and D contain a high proportion of individuals with a striking red color in the iris which is not observed in the other lineages, and several lineages can be distinguished by tadpole color pattern. Importantly, all lineages for which bioacoustic data are available differ in male advertisement calls, and in some cases strikingly so: for instance, the calls of lineages C and E are so divergent in all analyzed parameters that structural resemblances are barely recognizable (Table 2). The most conclusive evidence comes from occurrence in sympatry (sometimes syntopy as in A and D) of various lineages without admixture and maintaining the morphological, bioacoustic and genetic differences, thus satisfying even a conservative biological species criterion. Although not all possible pairwise comparisons are equally conclusive (e.g., bioacoustic data are lacking for lineage H), we are convinced that all eight lineages are evolutionarily independent and merit recognition as separate species. In the following, therefore, we revise *B. marojezensis* sensu stricto (lineage F) and formally name and describe the remaining seven lineages as new species.

Boophis marojezensis Glaw & Vences, 1994

Figures 6, 7

Identity. This species was discovered and scientifically named by Glaw and Vences (1994) based on a holotype specimen collected at low elevations in the Marojejy Massif. No vouchered call recording referring to the name-bearing type (holotype) had been recorded, and no DNA sequence was so far available from this specimen. Calls heard from near the type locality consisted of short and long notes whereas populations of the *B. marojezensis* complex from higher elevations in Marojejy emitted calls with a greater number of very short notes only. We here provide a 16S sequence of the type obtained via archival DNA extraction and Illumina shotgun sequences which confirms that the name *B. marojezensis* is to be assigned to the lineage found in Marojejy at low elevation as well as in a few other sites in northern Madagascar (lineage F), characterized by advertisement calls containing two different note types (long and short).

Vieites et al. (2009) and Perl et al. (2014) used the specimen FGZC 2857 as their reference individual for *B. marojezensis*. That individual belongs to a different lineage, the one we refer to here as lineage C, which was referred to as *B.* sp. Ca53 by Randrianiaina et al. (2012) based on specimens from Tsaratanana Strict Nature Reserve. A second specimen, ZSM 326/2000 from Vohidrazana, included by Vieites et al. (2009) as a deep conspecific lineage of *B. marojezensis*, is shown by our trees to belong to lineage D. The true *B. marojezensis* was referred to as *B.* sp. 25 by Vieites et al. (2009) and Randrianiaina et al. (2012), herein corresponding to lineage F.

Holotype. ZFMK 57401, by original designation. Type locality: "the Marojezy massif at low altitude, NE-Madagascar". A partial 16S sequence of the holotype is available from GenBank under accession number PQ278105.

Paratypes. One paratype: ZSM 567/1999 (previously ZFMK 57402), adult male, with same collection data as holotype.

Material examined. In addition to the type material, we examined ZSM 208/2022 (FGZC 6510), probably an adult male, collected on 24 March 2022 by J.M. Rafanoharana, H. Raherinjatovo and F. Glaw at Analanjirofo (near Simpona Lodge), Makira Reserve (15.19917°S, 49.62083°E, 410 m a.s.l.); and ZSM 250/2016 (FGZC 5439), adult male, collected on 12 August 2016 by F. Glaw, D. Prötzel, J. Forster, K. Glaw, and T. Glaw at Masoala, around the "Eco-Lodge chez Arol" (ca. 15.7122°S, 49.9640°E, ca. 21 m a.s.l.).

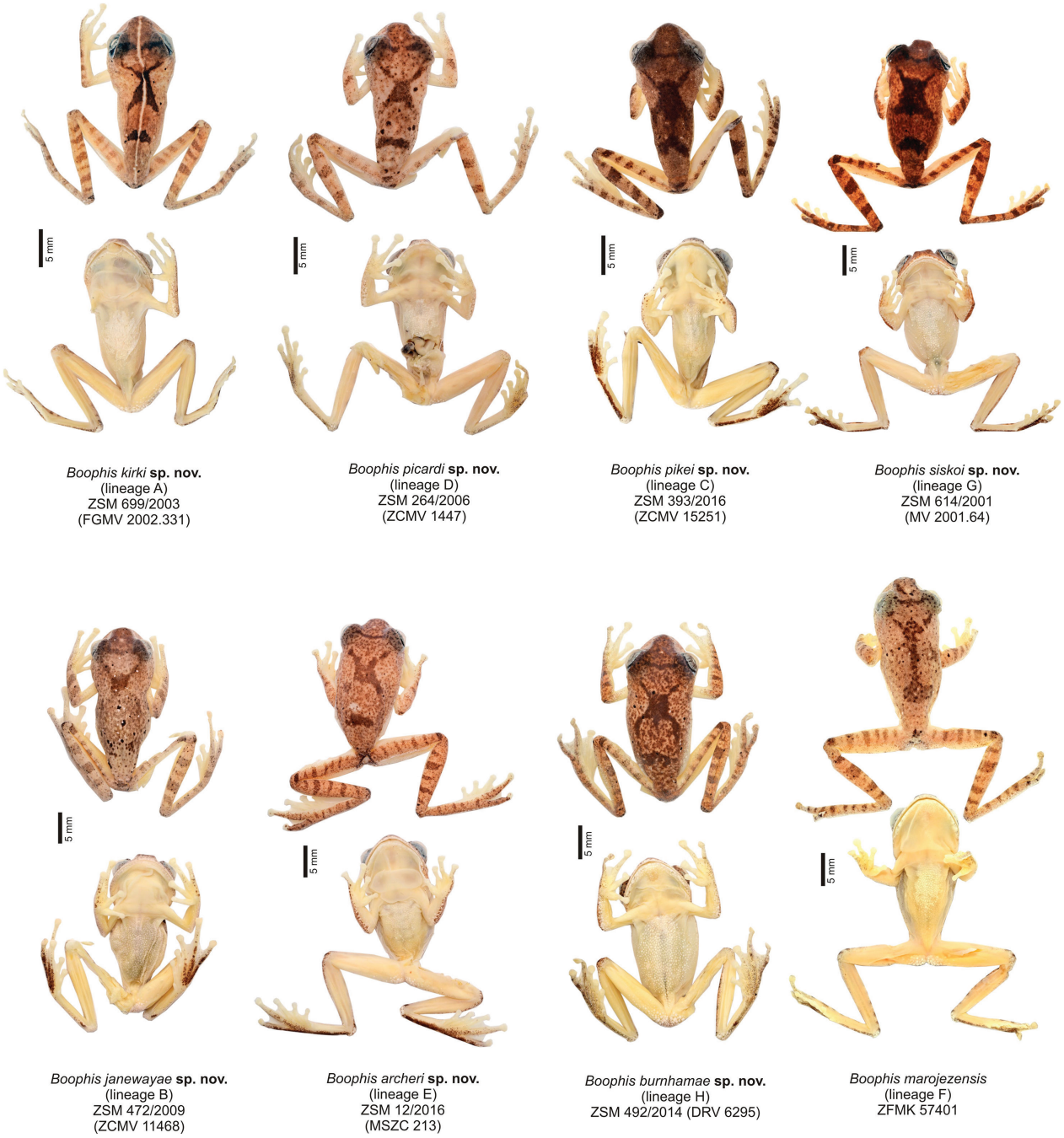

Figure 6. Preserved holotypes in dorsal and ventral views of the eight nominal species in the *Boophis marojezensis* complex described in this study.

Definition. A small treefrog assigned to the genus *Boophis*, subgenus *Boophis*, in the family Mantellidae based on its occurrence on Madagascar, presence of intercalary element between ultimate and penultimate phalanx of fingers and toes (verified by external examination), presence of webbing between fingers, presence of nuptial pads in males, and absence of femoral glands in males. Assigned to the *Boophis blommersae* group based on small body size (male SVL 20.0–25.7 mm), predominantly brownish dorsal coloration, absence of red color on webbing or ventral side of limbs, suctorial, stream-dwelling tadpoles, and molecular phylogenetic relationships. Within the *B. blommersae* group, defined by absence of dorsolateral bands, absence of red color in the outer iris area, and advertisement calls at a dominant frequency of 4118–4441 Hz, consisting of 7–8 notes of different length (short notes 15–51 ms; long whistling notes 142–259 ms). Also characterized by numerous diagnostic nucleotide positions in the mitochondrial 16S rRNA gene: MolD identified the following robust diagnostic nucleotide combination compared to all other species in the *B. marojezensis* complex (sites given relative to the full-length 16S sequence of *Mantella baroni*): "C" in the site 107, "G" in the site 162, "C" in the site 251.

Diagnosis. Within the *B. blommersae* group, distinguished from *B. blommersae* by calls containing multiple frequency-modulated whistles (vs. pulsed trills), and from

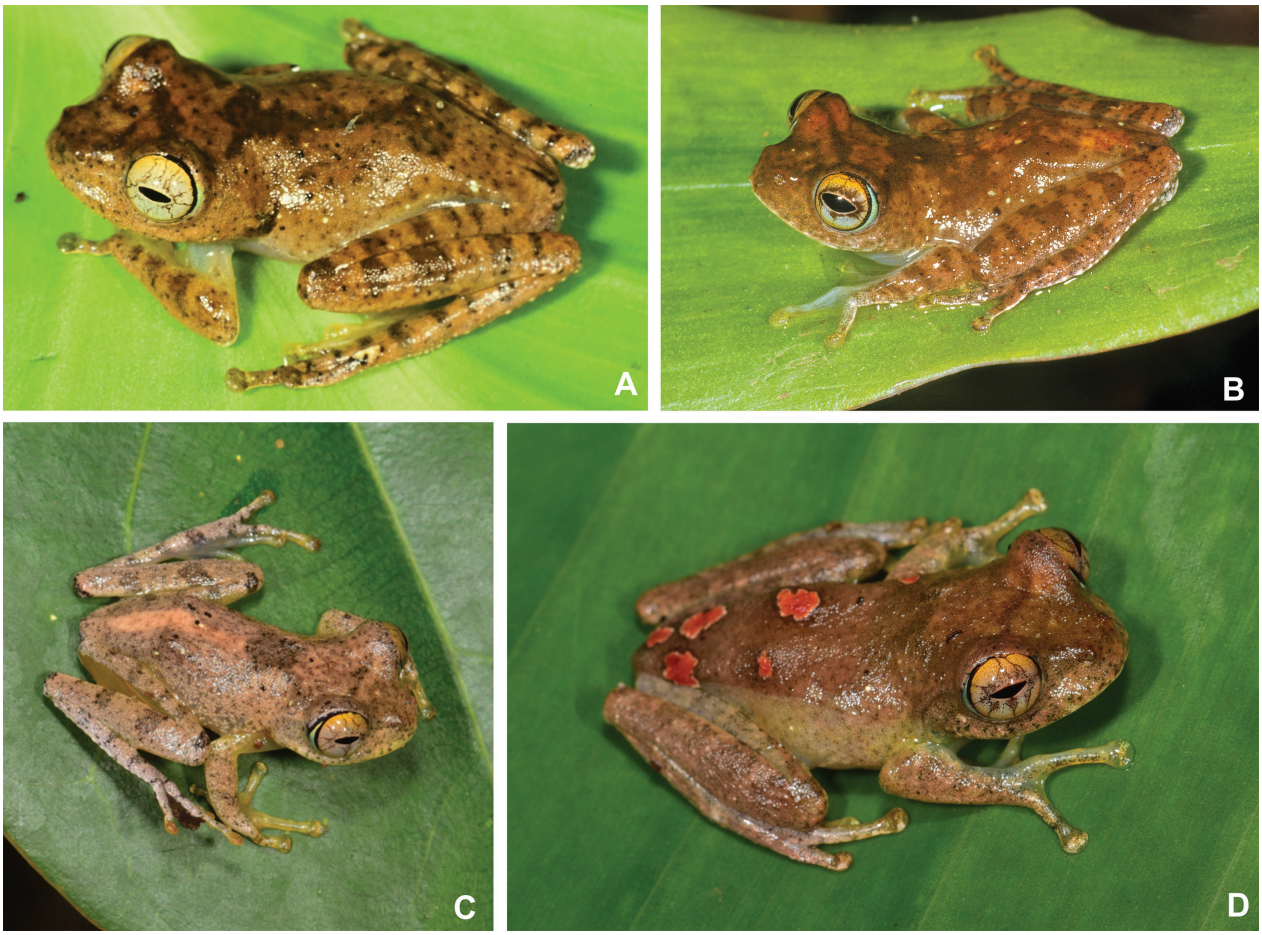

Figure 7. Individuals of *Boophis marojezensis* in life. **A** Male holotype (ZFMK 57401) from Marojejy (low elevation). **B** Male paratype (ZSM 567/1999, previously ZFMK 57402). **C** Individual from Masoala probably assignable to this species (not sequenced). **D** Male from Masoala (ZSM 250/2016, FGZC 5439).

B. vittatus by calls containing multiple frequency-modulated whistles (vs. series of short clicks), and absence of dorsolateral stripes (vs. presence). For a distinction from other species of the *B. marojezensis* complex described herein, see accounts of these new species below.

Tadpole. The tadpole of *B. marojezensis* (under the name *B. marojezensis* [Ca25]) was described and illustrated by Randrianiaina et al. (2012), based on the DNA barcoded specimen ZSM 1611/2007 (FGZC 2929; GenBank accession number FJ559146). As typical for all tadpoles of the group, the larvae belong to the "suctorial" ecomorphological guild. They have a large oral disk used to adhere to stones in fast-flowing water, a labial tooth row formula of 7(5-7)/3, and large numbers of oral papillae (222 marginal and 315 submarginal; without dorsal gap). They are characterized by a pattern of several rounded patches formed by condensation of spots on the posterior half of the tail musculature.

Natural history. An arboreal, nocturnal treefrog found in humid rainforests along fast flowing streams. Little is known of the ecology of the species. Many calling individuals of this species are sitting too high in trees to reach, or calling from perches where they are difficult to see and their calls are also often difficult to localize. Glaw et al. (2001) also reported calling activity in March, suggesting continuous reproductive activity throughout the rainy season.

Calls. Advertisement calls of *Boophis marojezensis* recorded at low elevation near a site known as "Camp 0", Marojejy National Park, on 26 November 2016 (22.8°C air temperature) consist of two different note types, namely calls starting with a short series of short, fast repeated notes, followed by three distinctly longer notes, which are separated by longer intervals. All notes are tonal in character, with long notes exhibiting a distinct upward frequency modulation, with a frequency shift comprising approximately 300 Hz. Amplitude across the entire call is slightly increasing, with the first short notes being relatively soft. Long notes have the maximum call energy in the middle of the note, but amplitude modulation within notes is somewhat irregular. Numerical parameters of two analyzed calls of different individuals are as follows: call duration 1129–1222 ms; notes/call 7–8; short note duration 30–51 ms (34.7 ± 7.5 ms); long note duration 147–259 ms (199.7 ± 44.7 ms); inter-note interval 19–179 ms (62.5 ± 55.9 ms); dominant frequency 4118–4347 Hz (4258 ± 82 Hz); prevalent bandwidth 3800–4500 Hz.

These calls are in general agreement with those recorded at Marojejy on 20 March 1994, which, however,

differ slightly in shorter inter-note intervals. Numerical parameters of two analyzed calls of the 1994 recording are as follows: call duration 764–1030 ms; notes/call 7–8; short note duration 15–49 ms (27.8 ± 10.6 ms); long note duration 142–231 ms (176.5 ± 38.1 ms); inter-note interval 22–61 ms (35.8 ± 14.1 ms); dominant frequency 4289–4441 Hz (4369 ± 64 Hz); prevalent bandwidth 4000–4700 Hz.

Distribution. According to the molecular data summarized herein, the species is known from (1) the type locality, the Marojejy Massif at low elevation (close to "Camp Mantella"), (2) a second site at Marojejy (sample THC 302, sequence downloaded from GenBank; collected at 14.4467°S, 49.8251°E, 225 m a.s.l. by T.R. Fulgence), (3) the Masoala Peninsula near the Eco-Lodge "Chez Arol", and (4) the Makira Reserve, at Analanjirofo near Simpona Lodge. *Boophis marojezensis* is a low-elevation species, known from 21–410 m a.s.l.

Boophis kirki sp. nov.

https://zoobank.org/DBC34BC5-2D84-41E6-927B-5D66358EFF9E

Lineage A = Ca51

Figures 6, 8

Identity. This species has previously been referred to as *B. marojezensis* [Ca51 JQ518198] = *B. marojezensis* [Ca51] by Randrianiaina et al. (2012) and *B.* sp. Ca51 in Hutter et al. (2018). It was included in *B. marojezensis* sensu lato by Glaw et al. (2001) and Glaw and Vences (2007) and not explicitly included or mentioned in the studies of Vieites et al. (2009) and Perl et al. (2014).

Holotype. ZSM 699/2003 (FGMV 2002.331), adult male, collected by F. Glaw, M. Puente, L. Raharivololoniaina, M. Thomas, and D.R. Vieites on 20 January 2003 at Kidonavo bridge, near Vohiparara, Ranomafana National Park (21.2167°S, 47.3667°E), ca. 1000 m a.s.l., Southern Central East of Madagascar.

Paratypes. ZSM 700/2003 (FGMV 2002.332), adult male, with same collection data as holotype. KU 336874 (CRH 17), adult male collected by C.R. Hutter, Z.F. Andriampenomanana, and S. Justin on 13 January 2014 at Valohoaka, Ranomafana National Park (21.2987°S, 47.4385°E, 1085 m a.s.l.). KU 336875 (CRH 18) adult male collected by C.R. Hutter, Z.F. Andriampenomanana, and S. Justin on 13 January 2014 at Valohoaka, Ranomafana National Park (21.2987°S, 47.4386°E, 1086 m a.s.l.). UADBA-CRH 15 (CRH 15), adult male collected by C.R. Hutter, Z.F. Andriampenomanana, and S. Justin on 13 January 2014 at Valohoaka, Ranomafana National Park (21.2973°S, 47.4389°E, 1067 m a.s.l.). KU 336876 (CRH 19), adult male, collected by C.R. Hutter, Z.F. Andriampenomanana, and S. Justin on 14 January 2014 at Valohoaka, Ranomafana National Park (21.2975°S, 47.4390°E, 1065 m a.s.l.). KU 336967 (CRH 20), adult male, collected by C.R. Hutter, Z.F. Andriampenomanana, and S. Justin on 14 January 2014 at Valohoaka, Ranomafana National Park (21.2975°S, 47.4390°E, 1065 m a.s.l.). KU 336877 (CRH 105), adult male, collected by C.R. Hutter, Z.F. Andriampenomanana, E. Rajery, and S. Justin on 28 January 2014 at Maharira, Ranomafana National Park (21.3399°S, 47.4108°E, 1272 m a.s.l.). KU 336880 (CRH 185), adult male, collected by C.R. Hutter, Z.F. Andriampenomanana, and S. Justin on 26 December 2013 at Valohoaka, Ranomafana National Park (21.2978°S, 47.4389°E, 1066 m a.s.l.). MRSN-A 2245 (FAZC 11467), adult, collected by F. Andreone between 30 January and 3 February 2003 at Farihimazava (= Farimazava) Forest, near Antoetra (ca. 20.8350°S, 47.3325°E, 1380–1420 m a.s.l.).

Additional material. The following specimens from Vohidrazana are not included in the paratype series due to their relatively high genetic divergence compared to specimens from the type locality: UADBA-CRH 430 (CRH 430), adult female, collected by C.R. Hutter, S.M. Lambert, and Z.F. Andriampenomanana on 3 January 2015 at Vohidrazana (18.9861°S, 48.5015°E, 1164 m a.s.l.). KU 340727 (CRH 449) and KU 340730 (CRH 455), two adult males, collected by C.R. Hutter, S.M. Lambert, and Z.F. Andriampenomanana on 4 January 2015 at Vohidrazana (18.9794°S, 48.5181°E, 1105 m a.s.l.).

Definition. A small treefrog assigned to the genus *Boophis*, subgenus *Boophis*, in the family Mantellidae based on occurrence on Madagascar, presence of intercalary element between ultimate and penultimate phalanx of fingers and toes (verified by external examination), presence of webbing between fingers, presence of nuptial pads in males, and absence of femoral glands in males. Assigned to the *Boophis blommersae* group based on small body size (male SVL 20.0–23.4 mm), predominantly brownish dorsal coloration, suctorial stream-dwelling tadpoles, and molecular phylogenetic relationships. Within the *B. blommersae* group, defined by absence of dorsolateral bands, presence of red color in the outer iris area, especially at the dorsal and ventral edges, in many specimens, and advertisement calls at 3499–5604 Hz, consisting of a series of 9–19 whistling notes of successively increasing durations of 54–105 ms. Also characterized by numerous diagnostic nucleotide positions in the mitochondrial 16S rRNA gene: MolD identified the following robust diagnostic nucleotide combination compared to all other species in the *B. marojezensis* complex (sites given relative to the full-length 16S sequence of *Mantella baroni*): "A" in the site 17, "C" in the site 50, "T" in the site 159, "A" in the site 179.

Diagnosis. Within the *B. blommersae* group, distinguished from *B. blommersae* by calls consisting of frequency-modulated whistles (vs. pulsed trills); and from *B. vittatus* by calls consisting of frequency-modulated whistles (vs. series of short clicks), and absence of dorso-

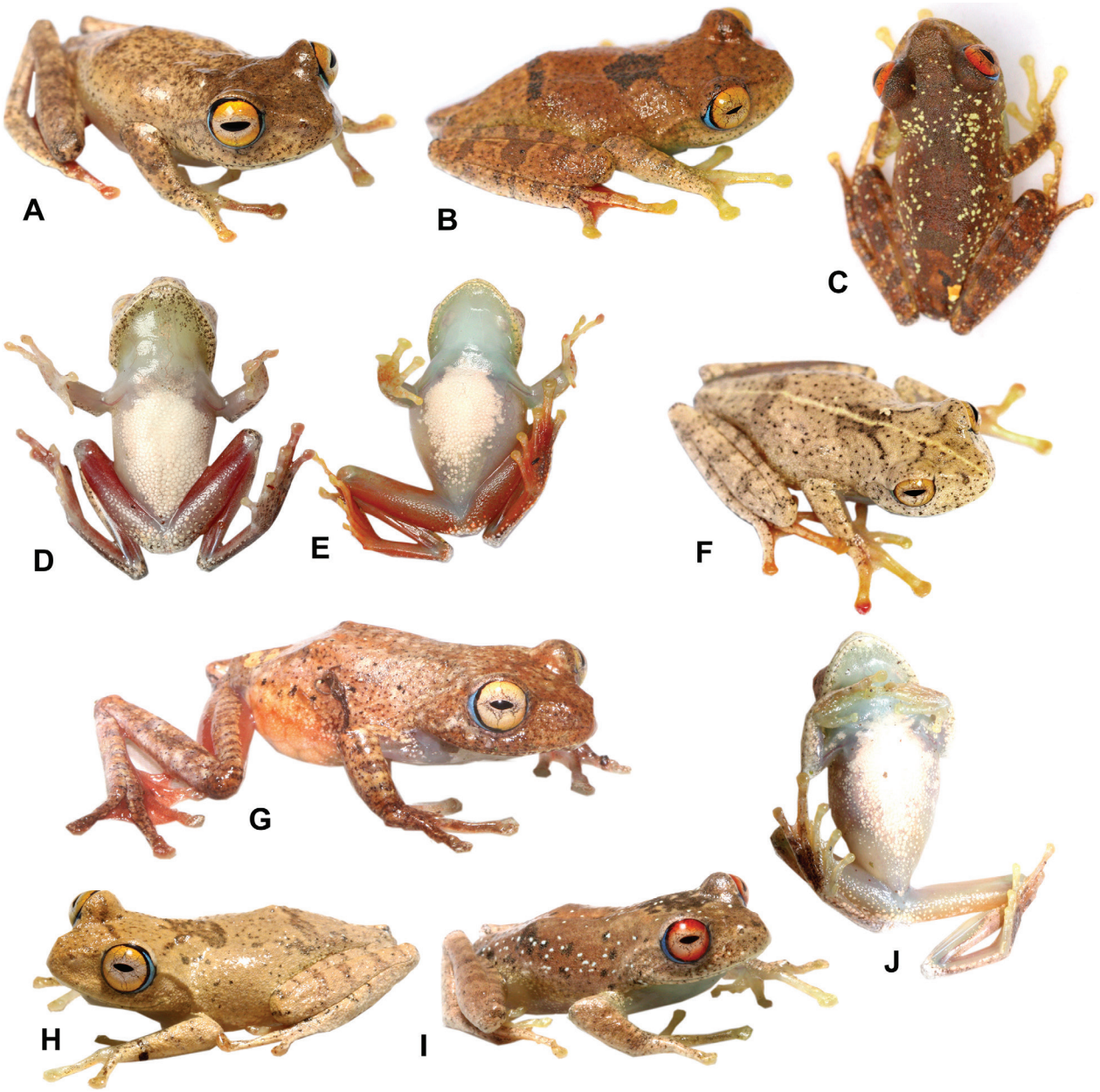

Figure 8. Individuals of *Boophis kirki* **sp. nov.** in life. **A**, **D** Male paratype KU 336967 (CRH 20) from Ranomafana in dorsolateral and ventral view. **B**, **E** Male paratype UADBA-CRH 15 (CRH 15) from Ranomafana in dorsolateral and ventral view. **C** Male paratype KU 336874 (CRH 17) from Ranomafana. **F** Male paratype KU 336875 (CRH 18) from Ranomafana. **G** Female (no type status) UADBA-CRH 430 (CRH 430) from Vohidrazana. **H** Male (no type status) KU 340727 (CRH 449) from Vohidrazana. **I**, **J** Male (no type status) KU 340730 (CRH 455) from Vohidrazana in dorsolateral and ventral view. Note the variation in iris color in both populations (Ranomafana and Vohidrazana), with some individual having red color in the outer iris area while others only have light orange color.

lateral stripes (vs. presence). Furthermore, distinguished from *B. marojezensis* by presence of red color in outer iris area in many specimens (vs. absence), and calls consisting of 9–19 whistling notes of successively increasing durations of 54–105 ms (vs. 7–8 notes, with short and long notes distinguishable). For a distinction from other species of the *B. marojezensis* complex described herein, see accounts of these new species below.

Description of the holotype. Adult male, in good state of preservation, SVL 23.4 mm, tongue and right forelimb removed as tissue samples for molecular analysis. Body slender; head wider than long, wider than body; snout rounded in dorsal view, truncate in lateral view; nostrils directed laterally, nearer to tip of snout than to eye; canthus rostralis indistinct, slightly concave in dorsal view, loreal region slightly concave; tympanum indistinct but recognizable, round, TD 50% of ED; supratympanic fold not recognizable; vomerine odontophores weakly developed, well-separated in two very small rounded aggregations, positioned posteromedial to choanae; choanae medium-sized, rounded; maxillary teeth present. Tongue removed. Arms slender, subarticular tubercles single, round; metacarpal tubercles not recognizable; fingers

weakly webbed and with lateral dermal fringes; webbing formula 1(traces), 2i(traces), 2e(traces), 3i(traces), 3e(1.75), 4(1); relative length of fingers 1<2<4<3 (finger 2 distinctly shorter than finger 4); finger discs enlarged, rounded; nuptial pads recognizable as unpigmented swelling on first finger. Hindlimbs slender; tibiotarsal articulation reaching nostril when hindlimb is adpressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle small, distinct, elongated; no outer metatarsal tubercle; toes broadly webbed; webbing formula 1(0.5), 2i(1), 2e(0), 3i(1), 3e(0.25), 4i(1.5), 4e(1.5), 5(0.25); relative length of toes 1<2<3<5<4; toe discs enlarged, rounded. Skin smooth on dorsal surfaces, throat, chest, and ventral surface of thighs, finely granular on belly; cloacal region surrounded by an area of distinct, large, white-colored granules.

In preservative, 20 years after collection (Fig. 6), dorsally reddish brown with a distinct and strongly contrasted dark brown hourglass-shaped marking on anterior part of the dorsum, and a dark brown transverse bar, somewhat chevron-shaped, on the posterior part of the dorsum. In addition, a few small dark brown and cream spots are scattered across the dorsum. A distinct light beige vertebral stripe runs from snout to cloaca and interrupts the dark markings. Limbs light brown with darker brown crossbands: 4–5 on forearm, 6–7 on shank, 6 on thigh. Ventrally cream, white on belly, with some dark pigment only on ventral side of feet. Color of holotype in life not recorded.

Variation. The paratype ZSM 700/2003 has a brown dorsal ground color with an irregular pattern of strongly contrasted small light markings, and neither vertebral stripe nor hourglass-patch. For variation of color in life in other paratypes, see Figure 8. A vertebral stripe as in the holotype is also observed in KU 336875, but not in the remaining specimens. Some individuals show a fine dotting with dark brown spots dorsally (e.g., KU 336967 and KU 336875, others have a dense spotting of small yellowish spots, mostly dorsolaterally (e.g., paratypes KU 336874 and KU 340730). Some specimens in life had relatively distinct red color ventrally on hindlimbs (Fig. 8D, E) while this was not apparent in others (e.g., Fig. 8J). The outer iris area was reddish in some specimens (KU 336874 from Ranomafana; KU 340730 from Vohidrazana) but only light orange in others (e.g., KU 336967). One female collected in January had light-colored oocytes visible through the ventral skin (UADBA-CRH 430; Fig. 8G). Iris periphery turquoise.

Etymology. Named after the fictional character Captain James T. Kirk, first portrayed by William Shatner in Gene Roddenberry's Star Trek (The Original Series), and also portrayed by Chris Pine in J.J. Abrams' Star Trek films.

Tadpole. The tadpole of this species (under the name *B. marojezensis* [Ca51]) was described and illustrated by Randrianiaina et al. (2012), based on the DNA barcoded specimen ZSM 267/2008 (ZCMV 3691; GenBank accession number JQ518198) from Ranomafana. As typical for all tadpoles of the group, the larvae belong to the "suctorial" ecomorphological guild. They have a large oral disk used to adhere to stones in fast-flowing water, a labial tooth row formula of 7(5-7)/3, and large numbers of oral papillae (297 marginal and 309 submarginal; without dorsal gap). They are characterized by absence of a lateral transparent area of the integument, tail muscle covered by reticulations mainly on the anterior half, and eyes situated between the anterior 3/10 and 4/10 of the body.

Natural history. An arboreal, nocturnal treefrog found in humid rainforests along fast flowing streams. Little is known of the ecology of the species. At Valohoaka in December 2013 and January 2014, male specimens were found calling sitting on leaves or branches, 1–2 m above the ground along streams. Additionally, calling males generally were perched 1 meter to occasionally several meters above the ground, at places that often could not be reached for observation.

Calls. Advertisement calls of *B. kirki* **sp. nov.** recorded at Ranomafana on 1 March 1996 (ca. 22–23°C air temperature) consist of multiple tonal notes of medium duration, repeated at regular intervals. In each call, the first note is always lower in dominant frequency (4640–4895 Hz) compared to subsequent notes (5528–5668 Hz). Moreover, the first 3–5 notes of each call have a lower relative amplitude than subsequent notes. Each note exhibits a distinct upward frequency modulation, encompassing a shift in dominant frequency range of about 700 Hz from the beginning to the end of the note. Within calls, notes tend to become slightly longer in duration from the beginning to the end of the call. Call energy is distributed in a narrow frequency band. Numerical parameters of four analyzed calls are as follows: call duration 2036–2355 ms (2170.0 ± 165.8 ms); notes/call 16–18 (16.8 ± 0.9); note duration 60–105 ms (75.7 ± 10.0 ms); inter-note interval 45–65 ms (54.0 ± 5.2 ms); note repetition rate within calls varies between ca. 6.5–8.2 notes/second; dominant frequency 5538–5604 Hz (5567 ± 34 Hz); prevalent bandwidth 4600–6100 Hz.

Calls recorded at Valohoaka, Ranomafana, on 12–13 January 2014 (air temperature not recorded) and corresponding to the voucher specimens KU 336874–336876 agree with the calls described above in overall character, although some shorter calls are evident in the recordings. Numerical parameters of seven analyzed calls of three individuals are as follows: call duration 1180–2630 ms (1944.7 ± 564.5 ms); notes/call 10–19 (14.5 ± 3.2); note duration 54–104 ms (74.4 ± 15.9 ms); inter-note interval 33–71 ms (59.8 ± 9.9 ms); dominant frequency 4981–5604 Hz (5371 ± 205 Hz); prevalent bandwidth 4000–5900 Hz.

Calls recorded at Vohidrazana, Andasibe region, on 4 January 2015 (air temperature 19°C) and corresponding to voucher specimen CRH 455 also agree in character with those from the Ranomafana region described above, but are lower in dominant frequency. Numerical parameters of four analyzed calls are as follows: call duration

1370–1537 ms (1427.3 ± 85.5 ms); notes/call 9–12 (10.0 ± 1.4); note duration 59–105 ms (86.5 ± 13.5 ms); inter-note interval 60–104 ms (72.8 ± 16.3 ms); dominant frequency 3499–4242 Hz (3998 ± 300 Hz); prevalent bandwidth 3300–4500 Hz.

Distribution. According to the molecular data summarized herein, the species is known from (1) the type locality, Vohiparara, and many other sites in Ranomafana National Park (Ambatolahy, Ambatovory, Fompohonina, Imaloka, Maharira, Mariavaratra, Ranomena, Sahateza, Samahalaotra, Valohoaka, Vatoharanana, Vohimanara), (2) from Antoetra (Farihimazava), based on barcoded specimen MRSN A2245 (Andreone et al. 2007); and (3) from Vohidrazana in the Andasibe region. The elevational range spans from mid elevations (e.g., 915 m a.s.l. at Ambatolahy) to relatively high elevations (1272 m a.s.l. at Maharira, up to 1420 m a.s.l. at Antoetra).

Boophis picardi sp. nov.

https://zoobank.org/AE0EE878-AD1A-4504-89C3-858BDA965D18

Lineage D

Figures 6, 9

Identity. This species has previously been referred to as *B.* sp. Ca68 in Hutter et al. (2018). It was included in *B. marojezensis* sensu lato by Glaw et al. (2001), Glaw and Vences (2007), Vieites et al. (2009), and Rosa et al. (2012), and not explicitly included or mentioned in the studies of Randrianiaina et al. (2012), and Perl et al. (2014). Adult specimens from Mandraka considered to represent *B. majori* by Blommers-Schlösser (1979b) are probably to be referred to this species.

Holotype. ZSM 264/2006 (ZCMV 1447), adult male, collected by D.R. Vieites, M. Vences, F. Rabemananjara, P. Bora, C. Weldon, and J. Patton on 7–8 February 2006 at An'Ala (18.9193°S, 48.4880°E, 889 m a.s.l.), Northern Central East of Madagascar.

Paratypes. ZSM 262/2006 (ZCMV 1436), ZSM 263/2006 (ZCMV 1437), ZSM 265/2006 (ZCMV 2364), three adult males with same collection data as holotype. ZSM 266/2006 (ZCMV 2403), ZSM 1969/2006 (ZCMV 1494), ZSM 1970/2006 (ZCMV 2417), three adult males with same collecting locality and collectors as holotype, but collected between 7–10 February 2006. ZSM 326/2000, adult male, collected by F. Glaw on 10 April 2000 at Vohidrazana (18.9658°S, 48.5103°E, 731 m a.s.l.). ZSM 189/2002 (FGMV 2001.1160), adult male, collected by M. Vences on 26–27 November 2001 at Vohidrazana (18.9658°S, 48.5103°E, 731 m a.s.l.). ZSM 1021/2003 (FGMV 2002.2359), adult male, collectors unknown, collected in 2003 at the type locality An'Ala (ca. 18.9193°S, 48.4880°E, ca. 880 m a.s.l.). KU 340631 (CRH 261), adult female, and KU 340641 (CRH 275), adult male, collected by C.R. Hutter, S.M. Lambert, Z.F. Andriampenomanana, and S. Justin on 10 December 2014 at Vohimana (18.9209°S, 48.5122°E, 787 m a.s.l.).

Definition. A small treefrog assigned to the genus *Boophis*, subgenus *Boophis*, in the family Mantellidae based on occurrence on Madagascar, presence of intercalary element between ultimate and penultimate phalanx of fingers and toes (verified by external examination), presence of webbing between fingers, presence of nuptial pads in males, and absence of femoral glands in males. Assigned to the *Boophis blommersae* group based on small body size (male SVL 21.3–23.2 mm), predominantly brownish dorsal coloration, absence of red color on webbing or ventral side of limbs of many specimens, calling males along streams, and molecular phylogenetic relationships. Within the *B. blommersae* group, defined by absence of dorsolateral bands, presence of distinct red color in outer iris area, especially its dorsal and ventral edges, in most specimens, and advertisement calls with high dominant frequencies of 4903–5819 Hz consisting of 17–25 whistling notes comprising multiple short (19–78 ms) and a few long notes (90–225 ms). Also characterized by numerous diagnostic nucleotide positions in the mitochondrial 16S rRNA gene: MolD identified the following robust diagnostic nucleotide combination compared to all other species in the *B. marojezensis* complex (sites given relative to the full-length 16S sequence of *Mantella baroni*): "G" in the site 177, "G" in the site 233, "C" in the site 314.

Diagnosis. Within the *B. blommersae* group, distinguished from *B. blommersae* by calls mainly consisting of frequency-modulated whistles (vs. pulsed trills); and from *B. vittatus* by calls mainly consisting of frequency-modulated whistles (vs. series of short clicks), and absence of dorsolateral stripes (vs. presence). Furthermore, distinguished from *B. marojezensis* by presence of red color in outer iris area in most specimens (vs. absence), and advertisement calls consisting of 17–25 notes (vs. 7–8 notes), and from *B. kirki* **sp. nov.** by advertisement calls consisting of two types of (short and long) notes (vs. notes of successively increasing duration). For a distinction from other species of the *B. marojezensis* complex described herein, see accounts of these new species below.

Description of the holotype. Adult male, in good state of preservation, SVL 23.0 mm, tissue from right thigh removed as tissue samples for molecular analysis and posterior venter cut open for parasitological examination. Body slender; head wider than long, wider than body; snout rounded in dorsal view, truncate in lateral view; nostrils directed laterally, about equidistant to tip of snout and eye; canthus rostralis indistinct, slightly concave in dorsal view, loreal region slightly concave; tympanum indistinct but recognizable, round, TD about 44% of ED; supratympanic fold very indistinct, largely straight; vomerine odontophores weakly developed, well-separated in two very small rounded aggregations,

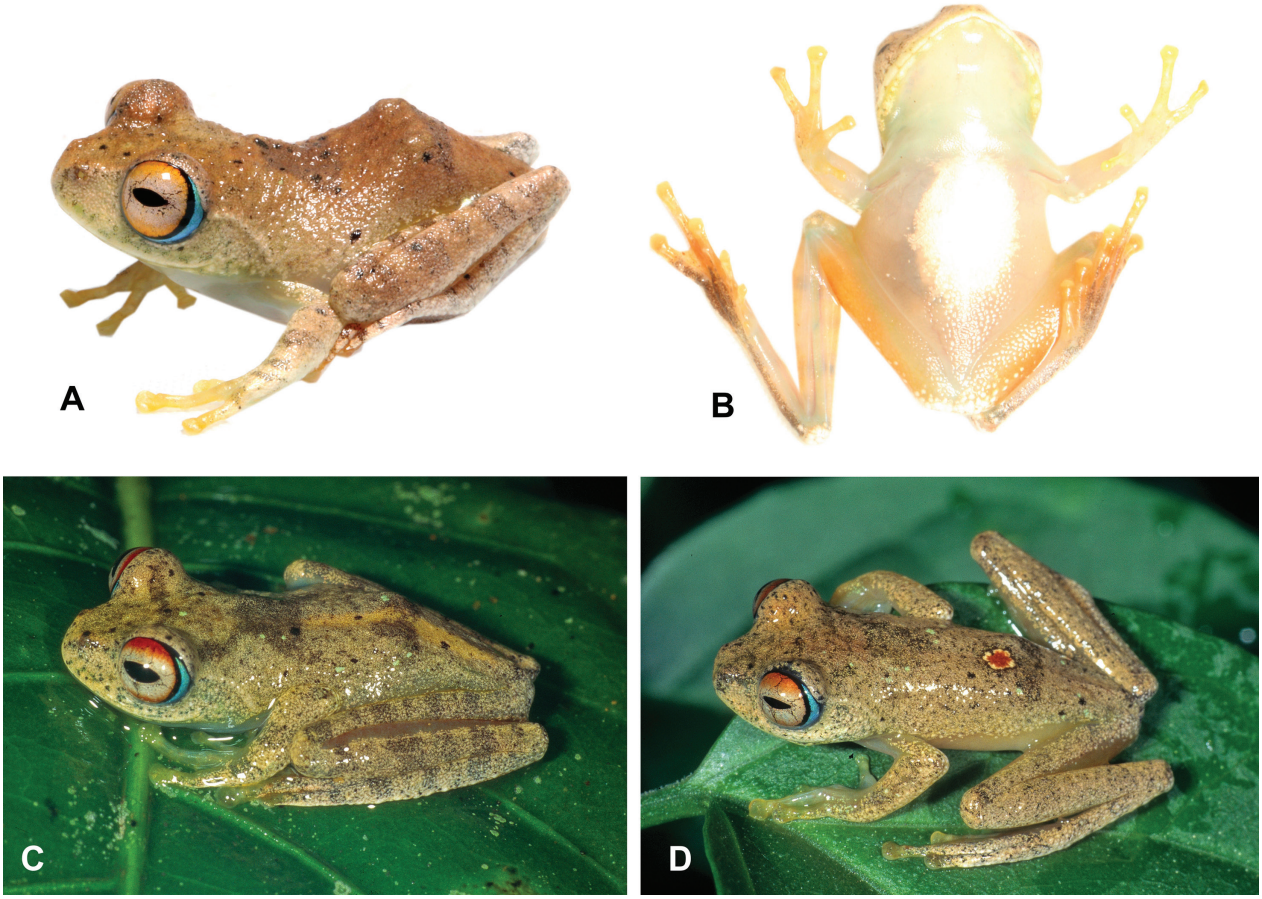

Figure 9. Individuals of *Boophis picardi* **sp. nov.** in life. **A, B** male paratype KU 340641 (CRH 275) from Vohimana in dorsolateral and ventral view. **C, D** male paratypes from An'Ala, not assignable to specific voucher specimens.

positioned posteromedial to choanae; choanae medium-sized, rounded; maxillary teeth present. Tongue ovoid, posteriorly bifid, free. Arms slender, forearms of slightly larger diameter, subarticular tubercles single, round; metacarpal tubercles not recognizable; fingers weakly webbed and with lateral dermal fringes; webbing formula 1(traces), 2i(traces), 2e(traces), 3i(traces), 3e(2), 4(1.5); relative length of fingers 1<2<4<3 (finger 2 distinctly shorter than finger 4); finger discs enlarged, rounded; nuptial pads indistinct, recognizable as unpigmented weak swelling on first finger. Hindlimbs slender; tibiotarsal articulation reaching nostril when hindlimb is adpressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle small, distinct, elongated; no outer metatarsal tubercle; toes broadly webbed; webbing formula 1(0.5), 2i(0.75), 2e(0.25), 3i(1), 3e(0), 4i(1.75), 4e(1.5), 5(0.5); relative length of toes 1<2<3<5<4; toe discs enlarged, rounded. Skin smooth on dorsal surfaces, throat, chest, and ventral surface of thighs, finely granular on belly; cloacal region surrounded by an area of distinct, large, white-colored granules.

In preservative, 17 years after collection (Fig. 6), dorsally reddish brown with a distinct and moderately contrasted dark brown hourglass marking on anterior part of the dorsum, and a dark brown transverse bar on the posterior part of the dorsum. Many dark spots of different sizes are scattered across the dorsum. Limbs light brown with a few rather poorly contrasted darker brown crossbands: 1–2 on forearm, 2–3 on shank, 2–3 on thigh. Ventrally cream, white on belly, with some dark pigment only on ventral side of feet. Color of holotype in life not recorded.

Variation. Several paratypes from An'Ala in preservative are characterized by a distinct dorsal hourglass pattern (plus additional patch posterior to it), particularly contrasted in ZSM 1970/2006 and 1969/2006. ZSM 268/2006 has a contrasted pinkish patch above the right eye, ZSM 326/2000 has a pinkish marking on the central dorsum and many small white-pinkish spots on the anterior dorsum, whereas ZSM 265/2006 has the dorsum covered with numerous larger pink patches (ca. 15 partly fused patches). ZSM 189/2002 from Vohidrazana features, in addition to the dorsal hourglass marking, a fine light vertebral line. In life, the dark dorsal pattern is often only weakly recognizable (Fig. 9). In one paratype (KU 340641), the hindlimbs have an orange tint in life but no deep red color is recognizable (Fig. 9B). The outer iris color can be deep red (Fig. 9C; see also Rosa et al. 2012) or light orange (Fig. 9A). Iris periphery light blue.

Etymology. Named after the fictional character Captain Jean-Luc Picard, first portrayed by Sir Patrick Stewart in Gene Roddenberry's Star Trek: The Next Generation, and later in Akiva Goldsman, Michael Chabon, Kirsten Beyer, and Alex Kurtzman's Star Trek: Picard.

Tadpoles. The tadpole of this species is unknown.

Natural history. An arboreal, nocturnal treefrog found in humid rainforests along fast flowing streams. Little is known of the ecology of the species. At Vohidrazana, male specimens were collected calling along a stream, from 1 meter to occasionally several meters above the ground. Many other calling males could be heard calling higher but were unable to be reached.

Calls. Advertisement calls of *B. picardi* **sp. nov.** recorded at An'Ala on 12 February 1995 (21.5°C air temperature) consist of two different note types, namely a series of rather short, quickly repeated notes, followed by 2–3 distinctly longer notes separated by slightly longer intervals. All notes are tonal in character and exhibit a distinct upward frequency modulation, with a frequency shift comprising 400–500 Hz in long notes and about half that in short notes. Amplitude across the entire call is increasing, with the first short notes being relatively soft, reaching maximum call energy at about one third of the call's duration. Within notes, no distinct amplitude modulation is recognizable. Numerical parameters of three analyzed calls of different individuals are as follows: call duration 1554–1832 ms (1673.3 ± 143.1 ms); notes/call 19–21 (20.3 ± 1.2); short note duration 24–53 ms (34.4 ± 6.6 ms); long note duration 112–200 ms (157.9 ± 26.8 ms); inter-note interval 25–96 ms (41.1 ± 17.5 ms); note repetition rate of short notes within the call vary around 16 calls/second; dominant frequency 4903–5444 Hz (5267 ± 152 Hz); prevalent bandwidth 4200–5600 Hz.

Calls of *B. picardi* **sp. nov.** recorded at Betampona on 30 October 2007, 22:30 h (air temperature 18°C) (from Rosa et al. 2011, 2012), are in general agreement with those described from An'Ala above. A slight difference is obvious for overall amplitude modulation across the call, with amplitude of notes slightly decreasing again after having reached maximum call energy at one third of the call's duration. Also, inter-note intervals are slightly longer compared to calls from An'Ala. Numerical parameters of two analyzed calls are as follows: call duration 1991–2000 ms; notes/call 17; short note duration 28–43 ms (32.8 ± 5.1 ms); long note duration 90–205 ms (140.8 ± 39.4 ms); inter-note interval 42–119 ms (64.6 ± 24.3 ms); note repetition rate of short notes within the call vary around 13 calls/second; dominant frequency 5442–5819 Hz (5667 ± 111 Hz); prevalent bandwidth 4500–6200 Hz.

The character of calls of *B. picardi* **sp. nov.** from Vohidrazana, recorded on 17 February 2001 (air temperature not recorded), generally agrees with those from An'Ala and Betampona described above, but Vohidrazana calls contain more long notes (6) following the short notes. Numerical parameters of two analyzed calls are as follows: call duration 2362–2388 ms; notes/call 24; short note duration 27–62 ms (37.8 ± 10.9 ms); long note duration 112–162 ms (139.0 ± 17.4 ms); inter-note interval 28–145 ms (57.5 ± 33.7 ms); note repetition rate of short notes within the call vary around 17 calls/second; dominant frequency 5292–5560 Hz (5417 ± 89 Hz); prevalent bandwidth 4900–5800 Hz.

Calls recorded at Vohidrazana, in December 2015 and January 2016 (air temperatures 17.9 and 20.3°C) and corresponding to call vouchers KU 342939 (CRH 971) and KU 342967 (CRH 1044) have the following numerical parameters (six calls analyzed): call duration 2010–2376 ms (2182.3 ± 119.7 ms); notes/call 20–25 (22.2 ± 1.8); short note duration 28–78 ms (38.0 ± 12.6 ms); long note duration 124–209 ms (163.3 ± 29.6 ms); inter-note interval 27–122 ms (55.8 ± 28.0 ms); note repetition rate of short notes within the call vary around 15 calls/second; dominant frequency 4971–5122 Hz (5060 ± 64 Hz); prevalent bandwidth 4200–5500 Hz.

Calls recorded at Mantadia, on 14 January 2017 (air temperature 19.1°C) and corresponding to the voucher specimen KU 347246 (CRH 1932) are also in agreement with the calls described above. Numerical parameters of three analyzed calls are as follows: call duration 2012–2330 ms (2165.0 ± 159.3 ms); notes/call 21–24 (23.0 ± 1.7); short note duration 19–75 ms (32.6 ± 14.0 ms); long note duration 123–225 ms (169.3 ± 33.9 ms); inter-note interval 35–89 ms (53.4 ± 17.7 ms); note repetition rate of short notes within the call vary around 15 calls/second; dominant frequency 5033–5388 Hz (5178 ± 119 Hz); prevalent bandwidth 4300–5600 Hz.

Distribution. According to the molecular data summarized herein, the species is known from several sites in the wider area around the village of Andasibe, i.e., (1) the type locality, An'Ala, (2) Vohidrazana, (3) Vohimana, and it also has been recorded from (4) Betampona (Sahambendrana and Sahabefoza sites, according to Rosa et al. 2012). The elevational range spans from 349 (Betampona, Sahabefoza) to 880 m a.s.l. (An'Ala).

Boophis pikei sp. nov.

https://zoobank.org/0D9A6696-0B4D-4BA8-9182-CC30A0EEBBE7

Lineage C

Figures 6, 10

Identity. This species has been previously referred to as *B. marojezensis* [Ca53 JQ518216] = *B. marojezensis* [Ca53] in Randrianiaina et al. (2012), and *B.* sp. Ca53 in Perl et al. (2014) and Hutter et al. (2018). This lineage also includes those specimens referred to *B. marojezensis* sensu stricto by Randrianiaina et al. (2012). It was included in *B. marojezensis* sensu lato by Glaw and Vences (2007) and Vieites et al. (2009), and not explicitly included or mentioned by Glaw et al. (2001).

Holotype. ZSM 393/2016 (ZCMV 15251), adult male, collected by M.D. Scherz, A. Rakotoarison, M. Bletz, M. Vences, and J. Razafindraibe on 18 November 2016 at Camp 3 "Simpona", Marojejy National Park (14.4366°S, 49.7434°E, 1325 m a.s.l.), North East of Madagascar.

Paratypes. ZSM 394/2016 (ZCMV 15259), adult male, same collection data as holotype. ZSM 1514/2012 (FGZC 3732), adult male, collected by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F.M. Ratsoavina, and A. Razafimanantsoa on 30 November 2012 in bamboo forest above a campsite on the Sorata massif (ca. 13.6752°S, 49.4410°E, ca. 1485 m a.s.l.). ZSM 107/2005 (FGZC 2853), ZSM 108/2005 (FGZC 2857), ZSM 109/2005 (FGZC 2871), ZSM 110/2005 (FGZC 2874) four adult males, collected by F. Glaw, M. Vences and R.D. Randrianiaina on 16 February 2005 at Camp 3 "Simpona", Marojejy National Park (14.4367°S, 49.7434°E, 1326 m a.s.l.).

Additional material. ZSM 1515/2012 (FGZC 3657), adult male (not DNA barcoded) collected by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F.M. Ratsoavina, and A. Razafimanantsoa on 28 November 2012 in Sorata (above campsite, ca. 13.6811°S, 49.4455°E, ca. 1398 m a.s.l.).

Definition. A small treefrog assigned to the genus *Boophis*, subgenus *Boophis*, in the family Mantellidae based on occurrence on Madagascar, presence of intercalary element between ultimate and penultimate phalanx of fingers and toes (verified by external examination), presence of webbing between fingers, presence of nuptial pads in males, and absence of femoral glands in males. Assigned to the *Boophis blommersae* group based on small body size (male SVL 21.4–25.0 mm), predominantly brownish dorsal coloration, absence of red color on webbing or ventral side of limbs, suctorial stream-dwelling tadpoles, and molecular phylogenetic relationships. Within the *B. blommersae* group, defined by absence of dorsolateral bands, absence of red color in outer iris area, and advertisement calls with high dominant frequencies of 5174–5507 Hz, consisting of fast series of 25–33 short whistling notes of 12–98 ms duration. Also characterized by numerous diagnostic nucleotide positions in the mitochondrial 16S rRNA gene: MolD identified the following robust diagnostic nucleotide combination compared to all other species in the *B. marojezensis* complex (sites given relative to the full-length 16S sequence of *Mantella baroni*): "C" in the site 86, "A" in the site 108, "G" in the site 254.

Diagnosis. Within the *B. blommersae* group, distinguished from *B. blommersae* by calls consisting of a fast series of short whistles (vs. pulsed trills); and from *B. vittatus* by calls consisting of a fast series of short whistles (vs. series of short clicks), and absence of dorsolateral stripes (vs. presence). Furthermore, distinguished from *B. marojezensis* by advertisement calls consisting of 25–33 notes (vs. 7–8 notes); from *B. kirki* **sp. nov.** by advertisement calls consisting of 25–33 notes (vs. 9–19 notes) and absence of red color in outer iris area (vs. presence in some specimens); and from *B. picardi* **sp. nov.** by advertisement calls consisting of 25–33 notes (vs. 17–25 notes) of maximum duration of 98 ms (vs. max. duration of 225 ms), and absence of red color in outer iris area (vs. distinct in many specimens). For a distinction from other species of the *B. marojezensis* complex described herein, see accounts of these new species below.

Description of the holotype. Adult male, in excellent state of preservation, SVL 22.3 mm, muscle tissue removed from right thigh for molecular analysis. Body slender; head slightly wider than long, much wider than body; snout rounded in dorsal view and rounded to slightly sloped in lateral view; nostrils directed laterally, equidistant to eye and tip of snout; canthus rostralis indistinct, slightly concave in dorsal view, loreal region slightly concave; tympanum indistinct but recognizable, round, TD 50% of ED; supratympanic fold poorly recognizable, mostly straight; vomerine odontophores weakly developed, well-separated in two very small rounded aggregations, positioned posteromedial to choanae; choanae medium-sized, rounded; maxillary teeth present. Tongue ovoid, posteriorly bifid, free. Arms slender, forearms of slightly larger diameter, subarticular tubercles single, round; metacarpal tubercles not recognizable; fingers weakly webbed and with lateral dermal fringes; webbing formula 1(traces), 2i(traces), 2e(traces), 3i(traces), 3e(2), 4(1.25); relative length of fingers 1<2<4<3 (finger 2 distinctly shorter than finger 4); finger discs enlarged, rounded; nuptial pads recognizable as unpigmented swelling on first finger. Hindlimbs slender; tibiotarsal articulation reaching beyond tip of snout when hindlimb is adpressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle small, distinct, elongated; no outer metatarsal tubercle; toes broadly webbed; webbing formula 1(1), 2i(1.5), 2e(0.5), 3i(1.75), 3e(0.5), 4i(1.75), 4e(1.75), 5(1); relative length of toes 1<2<3≤5<4; toe discs enlarged, rounded. Skin smooth on dorsal surfaces, throat, chest, and ventral surface of thighs, finely granular on belly; cloacal region surrounded by an area of distinct, large, white-colored granules.

In preservative, 7 years after collection (Fig. 6), dorsally brown with a distinct and moderately contrasted dark brown hourglass marking on anterior part of the dorsum, and a dark brown chevron-like transverse marking on the posterior part of the dorsum. An indistinct narrow dark transverse bar is visible between the eyes. Very few poorly contrasted light spots are scattered across the dorsum. Limbs light brown with distinct darker brown crossbands: about 3 on forearm, 3–5 on shank, 5 on thigh. Ventrally cream, white on belly, with dark pigment on ventral side of feet. In life (Fig. 10), similar but overall lighter and dorsal pattern less contrasted. Iris yellowish to beige, iris periphery turquoise.

Variation. A dark dorsal hourglass-marking in preservative is apparent in most paratypes and very extended, merging into a blackish patch covering most of the head in ZSM 109/2005. The female ZSM 107/2005 has only weakly contrasted traces of the hourglass-marking and is rather uniformly colored, with scattered light spots across the dorsum. ZSM 1514/2012 has a rather uniformly grayish dorsum and light flanks. In life, the dark dorsal markings are often only poorly contrasted or even absent (Fig. 10).

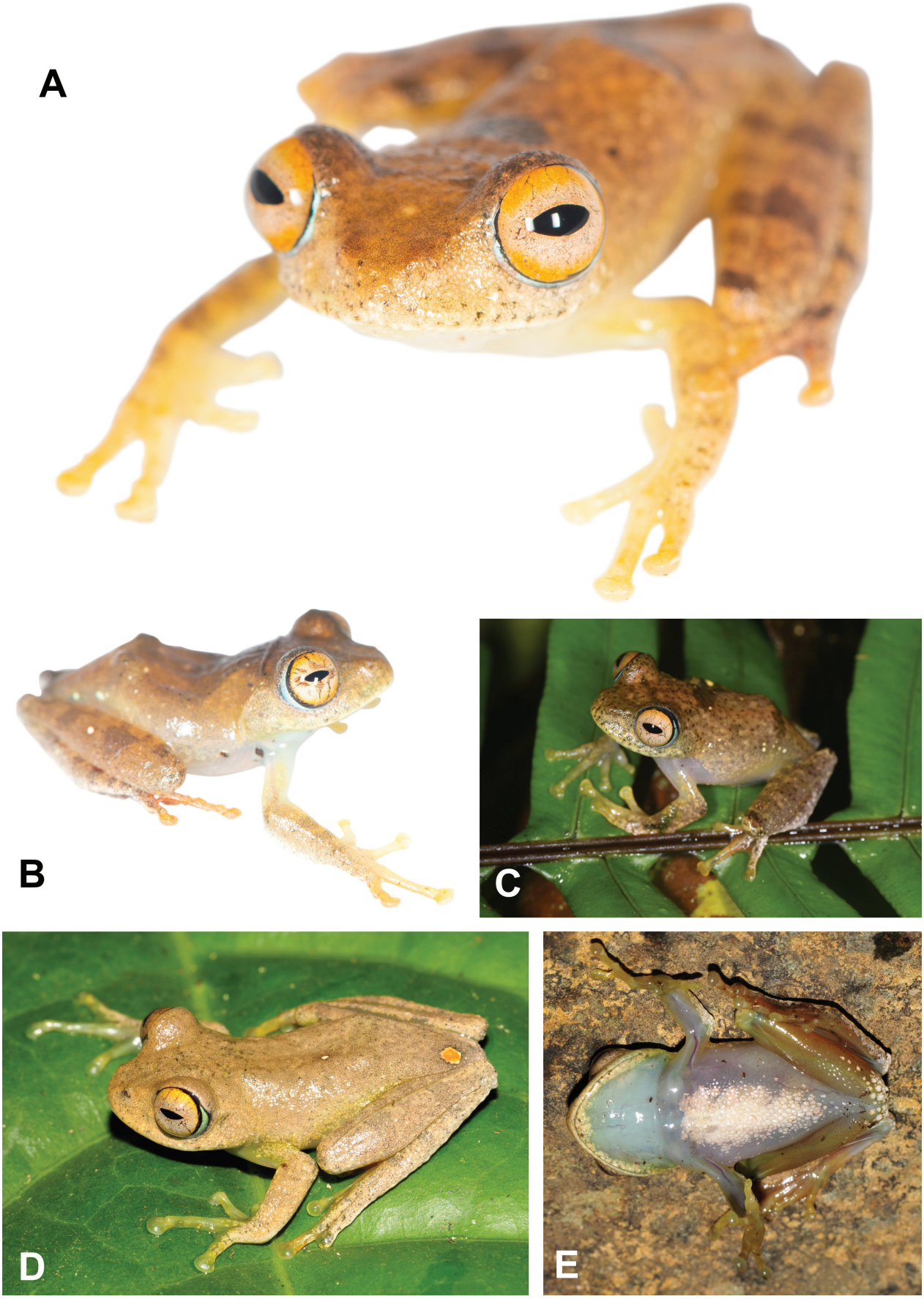

Figure 10. Individuals of *Boophis pikei* **sp. nov.** in life. **A** male paratype ZSM 394/2016 (ZCMV 15259) from Marojejy (Camp Simpona). **B** male holotype ZSM 393/2016 (ZCMV 15251) from Marojejy (Camp Simpona). **C** male from Marojejy (Camp Simpona), photo not assignable to a specific voucher specimen, photographed in 2016. **D, E** male paratype ZSM 1514/2012 (FGZC 3732) from Sorata.

Ventrally, in one photographed specimen, the belly is white, flanks unpigmented, and only traces of orange color are visible on hindlimbs (Fig. 10E). A sharply contrasted patch on the posterior-most dorsum is present in ZSM 1514/2012. The iris coloration can be rather uniform beige (e.g., ZSM 1514/2012) or include a light orange outer and beige inner iris area (ZSM 394/2016) (Fig. 10).

Etymology. Named after the fictional character Captain Christopher Pike, first portrayed by Sean Kenney and Jeffrey Hunter in Gene Roddenberry's Star Trek (The Original Series), and later portrayed by Anson Mount in Akiva Goldsman, Alex Kurtzman, and Jenny Lumet's Star Trek: Strange New Worlds.

Tadpole. Tadpoles of this species were described and illustrated under the names *B marojezensis* and *B. marojezensis* [Ca53] by Randrianiaina et al. (2012), based on the DNA barcoded specimens ZSM 1528/2007 (FGZC 2277; GenBank accession number JQ518196) from Marojejy National Park, and ZSM 573/2010 (ZCMV 13200; GenBank accession number JQ518216) from Tsaratanana, respectively. As typical for all tadpoles of the group, the larvae belong to the "suctorial" ecomorphological guild. They have a large oral disk used to adhere to stones in fast-flowing water, a labial tooth row formula of 7(5-7)/3, and large numbers of oral papillae (243–290 marginal and 452–660 submarginal; without dorsal gap). They are characterized by presence of a lateral transparent area of the integument.

Natural History. An arboreal, nocturnal treefrog found in humid rainforests along fast flowing streams. Little is known of the ecology of the species. In November 2016, a dense aggregation of calling males was found at night calling on low leaves and branches (ca. 1.2 m above the ground) between two narrow streams. At close range, the call is extremely loud.

Calls. Advertisement calls of *B. pikei* **sp. nov.** recorded at Camp Simpona, Marojejy, on 18 November 2016, 21:35 h (air temperature not recorded) consist of a high-pitched trill-like call composed of multiple very short tonal notes repeated at rather regular intervals and rapid succession. Within calls, usually the last 2–3 notes are slightly longer in duration when compared to leading notes (48–98 vs. 12–33 ms), and amplitude is modulated among notes, with call energy steadily increasing from the first to the last note of the call. Each note exhibits an upward frequency modulation, which is only very slightly expressed in the short notes, but more distinct in the last 2–3 notes of the call, comprising a shift in frequency ~100 Hz at maximum from beginning to the end of the note. Frequency modulation across the entire call may show some slight downward shift (Fig. 5). Numerical parameters of four analyzed calls from different individuals are as follows: call duration 921–1218 ms (1134.3 ± 142.6 ms); notes/call 25–33 (29.0 ± 3.4); note duration 12–98 ms (29.1 ± 22.6 ms); inter-note interval 17–31 ms (21.7 ± 4.1 ms); note repetition rate within calls varies between ca. 26–30 notes/second; dominant frequency 5174–5507 Hz (5367 ± 144 Hz); prevalent bandwidth 4800–5700 Hz.

Distribution. According to molecular data summarized herein, the species is known from (1) the type locality, higher elevations at the Marojejy Massif (around Camp Simpona), and (2) the Sorata Massif. Based on tadpoles, it also has been recorded (3) on the Tsaratanana Massif at a site called Antevialambazaha (14.1743°S, 48.9452°E,1699 m a.s.l.), by Randrianiaina et al. (2012). The elevational range of the species spans between 1325–1699 m a.s.l.

Boophis siskoi sp. nov.

https://zoobank.org/13ABB7F1-59EB-412D-865B-9AA63889F999

Lineage G

Figures 6, 11

Identity. This species has been previously referred to as *B. marojezensis* [Ca52 JQ518215] = *B. marojezensis* [Ca52] in Randrianiaina et al. (2012), and *B.* sp. Ca52 in Perl et al. (2014) and Hutter et al. (2018). It was included in *B. marojezensis* sensu lato in Glaw and Vences (2007) and not explicitly included or mentioned by Glaw et al. (2001) and Vieites et al. (2009).

Holotype. ZSM 614/2001 (FGMV 2001.64), adult male (call voucher) collected by F. Andreone, F. Mattioli, J. Randrianirina, and M. Vences on 3 February 2001 at Antsahamanara campsite, Manarikoba forest, Tsaratanana Massif (14.045°S, 48.784°E, ca. 1000 m a.s.l.), Sambirano region, Madagascar.

Paratypes. ZSM 615/2001 (FGMV 2001.71) and ZSM 616/2001 (FGMV 2001.80), two adult males with same collecting data as holotype but collected 4–9 February 2001 (ZSM 615/2001 not DNA barcoded). ZSM 10/2016 (MSZC 186), adult male, collected by M.D. Scherz, J. Borrell, L. Ball, T. Starnes, E. Razafimandimby, D.H. Nomenjanahary, and J. Rabearivony on 9 January 2016 at Ampotsidy, 15.7 km NNW of Bealanana (8.7 km NNW of Beandrarezona; 14.4276°S, 48.7223°E, 1320 m a.s.l.).

Definition. A small treefrog assigned to the genus *Boophis*, subgenus *Boophis*, in the family Mantellidae based on occurrence on Madagascar, presence of intercalary element between ultimate and penultimate phalanx of fingers and toes (verified by external examination), presence of webbing between fingers, presence of nuptial pads in males, and absence of femoral glands in males. Assigned to the *Boophis blommersae* group based on small body size (male SVL 25.0–27.2 mm), predominantly brownish dorsal coloration, absence of red color on webbing or ventral side of limbs, suctorial stream-dwelling tadpoles, and molecular phylogenetic relationships. Within the

B. blommersae group, defined by absence of dorsolateral bands, absence of red color in outer iris area, and advertisement calls with dominant frequencies of 4688–5332 Hz, consisting of 7–12 whistling notes of 44–220 ms duration, each with constant upward frequency modulation. Also characterized by numerous diagnostic nucleotide positions in the mitochondrial 16S rRNA gene: MolD identified the following robust diagnostic nucleotide combination compared to all other species in the *B. marojezensis* complex (sites given relative to the full-length 16S sequence of *Mantella baroni*): "G" in the site 109, "T" in the site 163, "A" in the site 233.

Diagnosis. Within the *B. blommersae* group, distinguished from *B. blommersae* by calls consisting of a series of whistles (vs. pulsed trills); and from *B. vittatus* by calls consisting of a series of whistles (vs. series of short clicks), and absence of dorsolateral stripes (vs. presence). Furthermore, distinguished from *B. marojezensis* by somewhat larger body size (male SVL 25.0–27.2 vs. 20.0–25.7 mm), advertisement calls consisting of notes of rather similar duration (vs. clear distinction of short and long notes); from *B. kirki* **sp. nov.** by larger body size (male SVL 25.0–27.2 vs. 20.0–23.4 mm), advertisement calls with notes of maximum duration of 220 ms (vs. max. duration of 105 ms) and with regularly ascending frequency modulation (vs. very steep initial frequency ascent in the beginning of each note, slowing down towards end of note), and absence of red color in outer iris area (vs. presence in some specimens); from *B. picardi* **sp. nov.** by larger body size (male SVL 25.0–27.2 vs. 21.3–23.2 mm), advertisement calls consisting of notes of rather similar duration (vs. clear distinction of short and long notes), and absence of red color in outer iris area (vs. distinct in many specimens); and from *B. pikei* **sp. nov.** by larger body size (male SVL 25.0–27.2 vs. 21.4–25.0 mm), and advertisement calls consisting of 7–12 notes (vs. 25–33 notes) of maximum duration of 220 ms (vs. max. duration of 98 ms). For a distinction from other species of the *B. marojezensis* complex described herein, see accounts of these new species below.

Description of the holotype. Adult male, in excellent state of preservation, SVL 25.8 mm, muscle tissue removed from left thigh for molecular analysis. Body moderately slender; head slightly wider than long and slightly wider than body; snout rounded to truncate in dorsal view, moderately rounded to sloping in lateral view; nostrils directed laterally, nearer to tip of snout than to eye; canthus rostralis rather weakly expressed, concave in dorsal view, loreal region slightly concave; tympanum rather distinct, round, TD 39% of ED; supratympanic fold distinct, slightly curved in its anterior and straight in its posterior half; vomerine odontophores weakly developed, well-separated in two very small rounded aggregations, positioned posteromedial to choanae; choanae small to medium-sized, rounded; maxillary teeth present. Tongue ovoid, posteriorly bifid, free. Arms slender, forearms of slightly larger diameter, subarticular tubercles single, round; metacarpal tubercles not recognizable; fingers weakly webbed and with lateral dermal fringes; webbing formula 1(traces), 2i(traces), 2e(traces), 3i(traces), 3e(2), 4(1); relative length of fingers 1<2<4<3 (finger 2 distinctly shorter than finger 4); finger discs enlarged, rounded; nuptial pads recognizable as unpigmented swelling on first finger. Hindlimbs slender; tibiotarsal articulation reaching beyond tip of snout when hindlimb is adpressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle small, distinct, elongated; no outer metatarsal tubercle; toes broadly webbed; webbing formula 1(0.5), 2i(0.75), 2e(0.5), 3i(1.25), 3e(0.5), 4i(2), 4e(2), 5(0.5); relative length of toes 1<2<3<5<4; toe discs enlarged, rounded. Skin smooth on dorsal surfaces, throat, chest, and ventral surface of thighs, finely granular on belly; cloacal region surrounded by an area of distinct, large, white-colored granules.

In preservative, 22 years after collection (Fig. 6), dorsally red brown with a distinct and moderately contrasted dark brown hourglass marking on anterior part of the dorsum, and a dark brown curved transverse bar on the posterior part of the dorsum. A narrow dark transverse bar is visible between the eyes. Dorsum with many poorly delimited and indistinct small dark spots. Limbs light brown with distinct darker brown crossbands: about 3 on forearm, 3–5 on shank, 5 on thigh. Ventrally cream, white on belly and with dark pigment on ventral side of feet. In life (Fig. 11), similar but dorsal color light brown rather than reddish brown. Outer iris color yellowish, inner iris color light brown, iris periphery turquoise.

Variation. All paratypes in preservative have the typical pattern of a dark hourglass-patch on the anterior and a transverse bar or inverted U-patch on the posterior dorsum. ZSM 616/2001 has a small pink dot on the central dorsum. In life, the dark dorsal markings are sometimes poorly contrasted (Fig. 11A).

Etymology. Named after the fictional character Captain Benjamin Sisko, first portrayed by Avery Brooks in Rick Berman and Michael Piller's Star Trek: Deep Space Nine.

Tadpole. The tadpole of this species (under the name *B. marojezensis* [Ca52]) was described and illustrated by Randrianiaina et al. (2012), based on the DNA barcoded specimen ZSM 541/2010 (ZCMV 13168; GenBank accession number JQ518215) from Ambinanitelo. As typical for all tadpoles of the group, the larvae belong to the "suctorial" ecomorphological guild. They have a large oral disk used to adhere to stones in fast-flowing water, a labial tooth row formula of 7(5-7)/3, and large numbers of oral papillae (258 marginal and 522 submarginal; without dorsal gap). They are characterized by presence of a (poorly recognizable) lateral transparent area of the integument, and absence of dark (melanophoric) pigment on the tail.

Natural history. An arboreal, nocturnal treefrog found in humid rainforests along fast flowing streams. Little is known of the ecology of the species. At Ampotsidy, this species was only encountered along a large river, and was not heard along narrower streams at higher elevations.

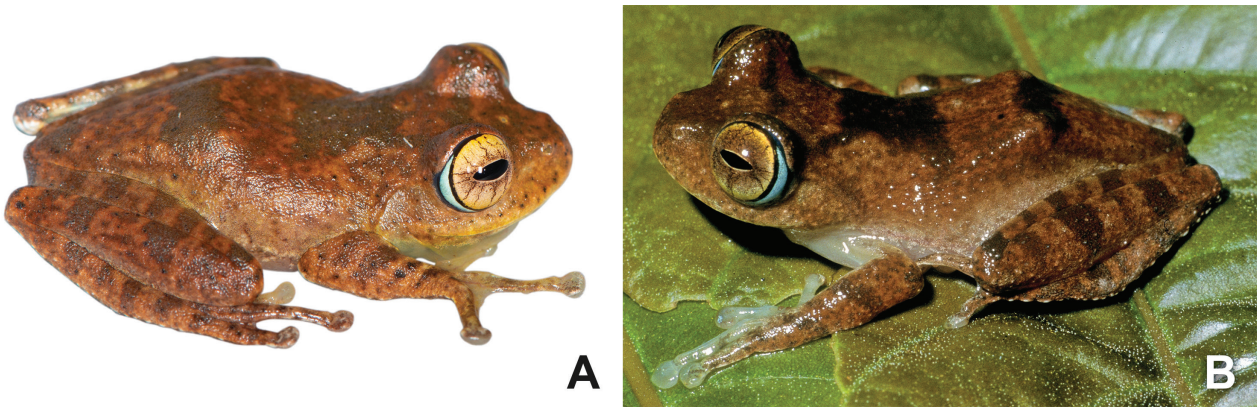

Figure 11. Individuals (adult males) of *Boophis siskoi* **sp. nov.** in life. **A** paratype ZSM 10/2016 (MSZC 186) from Ampotsidy. **B** holotype ZSM 614/2001 (FGMV 2001.64) from the Tsaratanana Massif (Antsahamanara campsite).

Calls. Advertisement calls of *B. siskoi* **sp. nov.** recorded at the type locality, Antsahamanara, Tsaratanana massif, on 3 February 2001 (24°C air temperature) consist of multiple notes (almost tonal in character, but with some irregular amplitude modulation) of variable duration, repeated at variable intervals. Within calls, note duration and inter-note intervals become longer from the beginning to the end of the call. Each note exhibits upward frequency modulation, which is most expressed in the last note (comprising a frequency shift of about 300 Hz) and much less in the first notes of the call. Overall frequency of the call exhibits a slight drop from the beginning to the end of about 200 Hz from the first notes compared to the last note (Fig. 5). However, in some calls the first 2–3 notes exhibit a lower frequency than subsequent ones. Each note is amplitude modulated with maximum call energy usually present somewhere in the first half of the note's duration. Numerical parameters of four analyzed calls are as follows: call duration 1549–2168 ms (1877.0 ± 311.2 ms); notes/call 10–12 (10.7 ± 1.2); note duration 44–220 ms (89.8 ± 44.5 ms); inter-note interval 41–162 ms (72.6 ± 31.7 ms); dominant frequency 4890–5332 Hz (5125 ± 222 Hz); prevalent bandwidth 4000–5600 Hz.

A call of *B. siskoi* **sp. nov.** recorded at Ampotsidy, on 13 January 2016 at 18:43 (air temperature not recorded), differs from the calls described from Tsaratanana by less variation in note duration and lack of a distinct overall frequency drop from the beginning to the end of the call (Fig 5). Numerical parameters of this analyzed call are as follows: call duration 1805 ms; notes/call 7; note duration 114–165 ms (144.3 ± 18.5 ms); inter-note interval 88–180 ms (125.7 ± 30.8 ms); dominant frequency 4688 Hz; prevalent bandwidth 3800–5200 Hz.

Distribution. According to molecular data summarized herein, the species is known from (1) the type locality, Antsahamanara campsite on the western versant of the Tsaratanana Massif, (2) Ambinanitelo forest (14.2254°S, 48.9635°E, 1182 m a.s.l.; Randrianiaina et al. 2012), and (3) Ampotsidy forest. The elevational range of the species spans between ca. 1000–1320 m a.s.l.

Boophis janewayae sp. nov.

https://zoobank.org/FBFBA6B3-5867-47E1-B673-ED249F-CFB042

Lineage B

Figures 6, 12

Identity. This species has been previously referred to as *B.* sp. aff. *marojezensis* [CaHM364579] by Rosa et al. (2012), and as *B. marojezensis* by Perl et al. (2014). It was not explicitly included or mentioned in the studies of Glaw et al. (2001), Glaw and Vences (2007), Vieites et al. (2009), Randrianiaina et al. (2012), and Hutter et al. (2018).

Holotype. ZSM 472/2009 (ZCMV 11468), adult male, collected by M. Vences, D.R. Vieites, F.M. Ratsoavina, R.D. Randrianiaina, E. Rajeriarison, T. Rajoafiarison, and J. Patton on 21 June 2009 at Angozongahy campsite, western side of Makira plateau (15.4370°S, 49.1186°E, 1009 m a.s.l.), North East of Madagascar.

Paratypes. ZSM 473/2009 (ZCMV 11486) and ZSM 474/2009 (ZCMV 11487), two adult males, with same collection data as holotype. ZSM 470/2009 (ZCMV 11270) and ZSM 471/2009 (ZCMV 11272), two adult males, collected by M. Vences, D.R. Vieites, F.M. Ratsoavina, R.D. Randrianiaina, E. Rajeriarison, T. Rajoafiarison, and J. Patton on 23–24 June 2009 at a campsite near the source of Fotsialanana river, western side of the Makira plateau (15.4668°S, 49.1289°E, 1067 m a.s.l.). ZSM 207/2022 (FGZC 6519), adult male, collected by J.M. Rafanoharana, H. Raherinjatovo, and F. Glaw on 24 March 2022 at Analanjirofo (near Simpona Lodge), Makira Reserve (15.1992°S, 49.6208°E, 410 m a.s.l.).

Definition. A small treefrog assigned to the genus *Boophis*, subgenus *Boophis*, in the family Mantellidae based on occurrence on Madagascar, presence of intercalary element between ultimate and penultimate phalanx of fingers and toes (verified by external examination), presence

of webbing between fingers, presence of nuptial pads in males, and absence of femoral glands in males. Assigned to the *Boophis blommersae* group based on small body size (male SVL 25.2–28.8 mm), predominantly brownish dorsal coloration, absence of red color on webbing or ventral side of limbs, calling males occurring along streams, and molecular phylogenetic relationships. Within the *B. blommersae* group, defined by absence of dorsolateral bands, absence of red color in outer iris area, and advertisement calls with low dominant frequencies of 2687–3404 Hz, consisting of 3–5 whistling notes of 238–604 ms duration. Also characterized by numerous diagnostic nucleotide positions in the mitochondrial 16S rRNA gene: MolD identified the following robust diagnostic nucleotide combination compared to all other species in the *B. marojezensis* complex (sites given relative to the full-length 16S sequence of *Mantella baroni*): "G" in the site 149, "T" in the site 190, "G" in the site 191.

Diagnosis. Within the *B. blommersae* group, distinguished from *B. blommersae* by calls consisting of a series of whistles (vs. pulsed trills); and from *B. vittatus* by calls consisting of a series of whistles (vs. series of short clicks), and absence of dorsolateral stripes (vs. presence). Furthermore, distinguished from *B. marojezensis* by advertisement calls with lower dominant frequency (2687–3404 vs. 4118–4441 Hz), with notes emitted at longer maximum inter-note intervals (639 vs. 179 ms), and larger body size (male SVL 25.2–28.8 vs. 20.0–25.7 mm); from *B. kirki* **sp. nov.** by advertisement calls with lower dominant frequency (2687–3404 Hz vs. 3499–5604 Hz), longer note duration (238–604 ms vs. 54–105 ms), larger male body size (SVL 25.2–28.8 vs. 20.0–23.4 mm), and absence of red color in outer iris area (vs. presence in some specimens); from *B. picardi* **sp. nov.** by advertisement calls with lower dominant frequency (2687–3404 Hz vs. 4903–5819 Hz), consisting of 3–5 notes (vs. 17–25 notes), longer note duration (238–604 ms vs. 19–225 ms), larger body size (male SVL 25.2–28.8 vs. 21.3–23.2 mm), and absence of red color in outer iris area (vs. distinct in many specimens); from *B. pikei* **sp. nov.** by advertisement calls consisting of 3–5 notes (vs. 25–33 notes) of 238–604 ms note duration (vs. max. duration of 98 ms), with lower dominant frequency (2687–3404 vs. 5174–5507 Hz), and larger body size (male SVL 25.2–28.8 vs. 21.4–25.0 mm); and from *B. siskoi* **sp. nov.** by advertisement calls consisting of 3–5 notes (vs. 7–12 notes) of 238–604 ms note duration (vs. max. duration of 220 ms) and with lower dominant frequency (2687–3404 vs. 4688–5332 Hz). For a distinction from other species of the *B. marojezensis* complex described herein, see accounts of these new species below.

Description of the holotype. Adult male, in excellent state of preservation, SVL 27.4 mm, muscle tissue removed from right thigh for molecular analysis. Body moderately slender; head slightly wider than long and slightly wider than body; snout rounded in dorsal and lateral views; nostrils directed laterally, about equidistant between tip of snout and eye; canthus rostralis distinct, concave in dorsal view, loreal region slightly concave; tympanum indistinct, round, TD about 54% of ED; supratympanic fold not recognizable (traces posterior to tympanum); vomerine odontophores weakly developed, well-separated in two very small rounded aggregations, positioned posteromedial to choanae; choanae medium-sized, rounded; maxillary teeth present. Tongue ovoid, posteriorly bifid, free. Arms slender, forearms of slightly larger diameter, subarticular tubercles single, round; metacarpal tubercles not recognizable; fingers weakly webbed and with lateral dermal fringes; webbing formula 1(traces), 2i(traces), 2e(traces), 3i(traces), 3e(2), 4(1.5); relative length of fingers 1<2<4<3 (finger 2 distinctly shorter than finger 4); finger discs enlarged, rounded; nuptial pads recognizable as unpigmented swelling on first finger. Hindlimbs slender; tibiotarsal articulation reaching between nostril and tip of snout when hindlimb is adpressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle small, distinct, elongated; no outer metatarsal tubercle; toes broadly webbed; webbing formula 1(0), 2i(0.5), 2e(0.25), 3i(1.25), 3e(0.25), 4i(1.75), 4e(1.75), 5(0.5); relative length of toes 1<2<3<5<4; toe discs enlarged, rounded. Skin smooth on dorsal surfaces, throat, chest, and ventral surface of thighs, finely granular on belly; cloacal region surrounded by an area of distinct, large, white-colored granules.

In preservative, 14 years after collection (Fig. 6), dorsally light brown with an indistinct and poorly contrasted brown hourglass marking on anterior part of the dorsum. No dark transverse bar is visible on the posterior part of the dorsum, but a comparatively wide dark transverse bar is present between the eyes. Dorsum with an irregular pattern of dark brown, black and whitish small spots, many of which are poorly contrasted. Limbs light brown with darker brown crossbands: 3–4 on forearm, about 5 on shank, about 5 on thigh. Ventrally cream, white on belly and with dark pigment on ventral side of feet. In life (Fig. 12), similar but the dorsal hourglass pattern almost not recognizable, crossbands on limbs poorly contrasted, but a few black and white dorsal spots well visible and contrasted. Outer iris color yellowish, inner iris color beige, iris periphery turquoise.

Variation. Two paratypes from Makira West (ZSM 470/2009 and 471/2009) in preservative are characterized by a contrasted dark hourglass-marking on the anterior dorsum and an inverted U-shaped marking on the posterior dorsum. ZSM 473/2009 has only a poorly contrasted hourglass-marking but features a light vertebral line. ZSM 474/2009 has a pattern of irregular fine light spots.

Etymology. Named after the fictional character Captain Kathryn Janeway, first portrayed by Kate Mulgrew in Rick Berman, Michael Piller, and Jeri Taylor's Star Trek: Voyager.

Tadpole. The tadpole of this species is unknown.

Natural history. An arboreal, nocturnal treefrog. Little is known of the ecology of the species. It has been found in

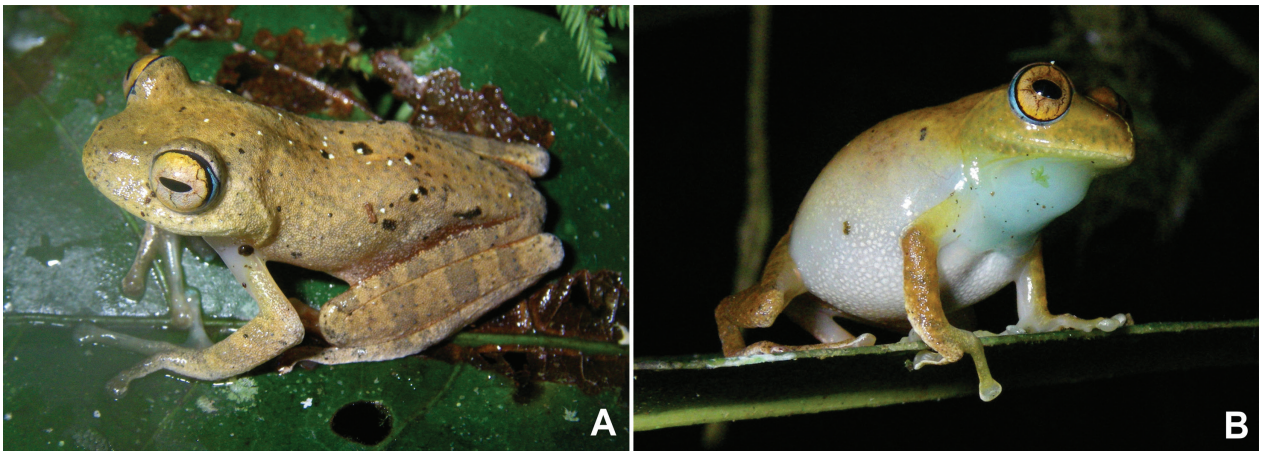

Figure 12. Individuals of *Boophis janewayae* **sp. nov.** in life. **A** male holotype ZSM 472/2009 (ZCMV 11468) from Angozongahy campsite, western side of Makira plateau. **B** calling male (with deflated vocal sac, during a pause between calls) photographed in the field at night, at the type locality.

humid rainforests along relatively slow-moving streams. Calling males were perched 1.5 to 2 m above the ground on leaves.

Calls. Advertisement calls of *B. janewayae* **sp. nov.** recorded from the holotype at Angozongahy, Makira area, on 21 June 2009 (air temperature not recorded) consist of a series of comparatively long tonal notes of variable duration, emitted at somewhat irregular intervals. Within calls, the first (and sometimes also the last) note is longest in duration. Amplitude is modulated within notes, with maximum call energy being present at each note's end. Each note exhibits an upward frequency modulation comprising a shift in frequency of ~400 Hz at maximum from beginning to the end of the note. Numerical parameters of two analyzed calls are as follows: call duration 2358–2846 ms; notes/call 4–5; note duration 238–604 ms (347.6 ± 118.2 ms); inter-note interval 206–639 ms (304.3 ± 152.5 ms); dominant frequency 2687–2996 Hz (2865 ± 111 Hz); prevalent bandwidth 2100–3300 Hz; harmonic frequency bands are evident at around 6000, 9000, and 12000 Hz.

Calls of *B. janewayae* **sp. nov.** recorded at Betampona, on 15 November 2007, 23:43 h (21°C air temperature) (from Rosa et al. 2011, 2012), generally agree in character with those described above from Angozongahy, but differ slightly in showing more regular inter-note intervals and slightly higher dominant frequency. Numerical parameters of two analyzed calls are as follows: call duration 1571–2064 ms; notes/call 3–4; note duration 276–536 ms (365.4 ± 116.1 ms); inter-note interval 141–278 ms (220.2 ± 55.8 ms); dominant frequency 3036–3404 Hz (3231 ± 135 Hz); prevalent bandwidth 2500–3600 Hz; harmonic frequency bands are evident at around 6340, 9500, and 12630 Hz.

Distribution. According to molecular data summarized herein, the species is known from (1) the type locality, western side of the Makira Reserve (Angozongahy and Fotsialanana), (2) the eastern side of the Makira Reserve (around "Simpona Lodge"), and (3) Betampona Reserve (sites: Sahambendrana, Sahabefoza, Sahaindrana, Vohitsivalana; Rosa et al. 2012). The elevational occurrence of the species spans from 349 m a.s.l. (Sahaindrana site in Betampona; Rosa et al. 2012) to 1067 m a.s.l. (Fotsialanana source, Makira).

Boophis archeri sp. nov.

https://zoobank.org/9AF5AB27-BC1C-43D5-946D-59F58DA002B5

Lineage E

Figures 6, 13

Identity. This species has been newly discovered in this study and has not been included in any of the previous studies including *B. marojezensis* and allied candidate species (e.g., Glaw et al. 2001; Glaw and Vences 2007; Vieites et al. 2009; Randrianiaina et al. 2012; Perl et al. 2014; Hutter et al. 2018).

Holotype. ZSM 12/2016 (MSZC 213), adult male, collected by M.D. Scherz and M. Rakotondratisma on 15 January 2016 at Bevitagnono forest, 33.1 km SW of Bealanana on the road RN31 (14.7387°S, 48.5170°E, 1016 m a.s.l.), North West of Madagascar.

Paratypes. ZSM 11/2016 (MSZC 198), adult male, collected by M.D. Scherz and M. Rakotondratsima on 14 January 2016 at Andranonafindra forest, 30 km SW of Bealanana on the road RN31 (14.7360°S, 48.5481°E, 1169 m a.s.l.); ZSM 13/2016 (MSZC 243), adult female, collected by M.D. Scherz and M. Rakotondratsima on 17 January 2016 at Andranonafindra forest, 30 km SW of Bealanana on the road RN31 (14.7360°S, 48.5489°E, 1138 m a.s.l.).

Definition. A small treefrog assigned to the genus *Boophis*, subgenus *Boophis*, in the family Mantellidae based on oc-

currence on Madagascar, presence of intercalary element between ultimate and penultimate phalanx of fingers and toes (verified by external examination), presence of webbing between fingers, presence of nuptial pads in males, and absence of femoral glands in males. Assigned to the *Boophis blommersae* group based on small body size (male SVL 25.0–25.3 mm), predominantly brownish dorsal coloration, absence of red color on webbing or ventral side of limbs, males calling along streams, and molecular phylogenetic relationships. Within the *B. blommersae* group, defined by absence of dorsolateral bands, absence of red color in outer iris area, and advertisement calls with dominant frequencies of 4130–4799 Hz, consisting of a series of 3–6 whistling notes of 158–308 ms duration and strong frequency modulation (frequency ascending and then descending in each note). Also characterized by numerous diagnostic nucleotide positions in the mitochondrial 16S rRNA gene: MolD identified the following robust diagnostic nucleotide combination compared to all other species in the *B. marojezensis* complex (sites given relative to the full-length 16S sequence of *Mantella baroni*): "D" in the site 253, "T" in the site 277, "A" in the site 303.

Diagnosis. Within the *B. blommersae* group, distinguished from *B. blommersae* by calls consisting of a series of whistles (vs. pulsed trills); and from *B. vittatus* by calls consisting of a series of whistles (vs. series of short clicks), and absence of dorsolateral stripes (vs. presence). Furthermore, distinguished from *B. marojezensis* by advertisement calls consisting of notes of rather similar duration (vs. clear distinction of short and long notes), and minimum note duration of 158 ms (vs. 15 ms); from *B. kirki* **sp. nov.** by advertisement calls consisting of 3–6 notes (vs. 9–19 notes) and longer note duration (158–308 ms vs. 54–105 ms), and absence of red color in outer iris area (vs. presence in some specimens); from *B. picardi* **sp. nov.** by advertisement calls consisting of notes of rather similar duration (vs. clear distinction of short and long notes), consisting of 3–6 notes (vs. 17–25 notes), minimum note duration 158 ms (vs. 19 ms), and absence of red color in outer iris area (vs. distinct in many specimens); from *B. pikei* **sp. nov.** by advertisement calls consisting of 3–6 notes (vs. 25–33 notes) of 158–308 ms note duration (vs. max. duration of 98 ms); from *B. siskoi* **sp. nov.** by advertisement calls consisting of 3–6 notes (vs. 7–12 notes), with each note strongly frequency-modulated with an initial ascent and final descent of frequency (vs. regularly ascending frequency); and from *B. janewayae* **sp. nov.** by advertisement calls with each note strongly frequency-modulated with an initial ascent and final descent of frequency (vs. regularly ascending frequency), and higher dominant frequency (4130–4799 vs. 2687–3404 Hz). For a distinction from other species of the *B. marojezensis* complex described herein, see accounts of these new species below.

Description of the holotype. Adult male, in excellent state of preservation, SVL 25.3 mm, muscle tissue removed from left thigh for molecular analysis. Body moderately slender; head slightly wider than long, as wide as body; snout rounded in dorsal view, sloped to rounded in lateral view; nostrils directed laterally, nearer to eye than to tip of snout; canthus rostralis indistinct, slightly concave in dorsal view, loreal region slightly concave; tympanum very indistinct, estimated TD about 43% of ED; supratympanic fold poorly recognizable, slightly curved in its anterior and more straight in its posterior half; vomerine odontophores weakly developed, well-separated in two small rounded aggregations, positioned posteromedial to choanae; choanae medium-sized, rounded to ovoid; maxillary teeth present. Tongue ovoid, posteriorly bifid, free. Arms slender, forearms of slightly larger diameter, subarticular tubercles single, round; metacarpal tubercles not recognizable; fingers weakly webbed and with lateral dermal fringes; webbing formula 1(traces), 2i(traces), 2e(traces), 3i(traces), 3e(2), 4(1.5); relative length of fingers 1<2<4<3 (finger 2 distinctly shorter than finger 4); finger discs enlarged, rounded; nuptial pads recognizable as unpigmented swelling on first finger. Hindlimbs slender; tibiotarsal articulation reaching anterior edge of eye when hindlimb is adpressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle small, distinct, elongated; no outer metatarsal tubercle; toes broadly webbed; webbing formula 1(0.25), 2i(0.75), 2e(0), 3i(1.25), 3e(0), 4i(1.75), 4e(1.75), 5(0.25); relative length of toes 1<2<3<5<4; toe discs enlarged, rounded. Skin smooth on dorsal surfaces, throat, chest, and ventral surface of thighs, finely granular on belly; cloacal region surrounded by an area of distinct, large, white-colored granules.

In preservative, 7 years after collection (Fig. 6), dorsally light reddish brown with a moderately contrasted but incomplete and somewhat discontinuous brown hourglass marking on anterior part of the dorsum, a broad brown transverse bar on the posterior part of the dorsum, and a narrow dark transverse bar between the eyes. Dorsum densely spotted with poorly contrasted small brown spots. Limbs light brown with darker brown crossbands: about 4 poorly marked crossbands on forearm, 5 on shank, 7–8 on thigh. Ventrally cream, white on belly and with dark pigment on ventral side of feet. In life (Fig. 13), dorsally cream and all dark dorsal elements poorly contrasted. Iris rather uniformly beige, iris periphery turquoise.

Variation. The female ZSM 13/2016 in preservative has a pattern of fine blackish spots across the dorsum, in addition to a moderately contrasted hourglass-patch on the anterior dorsum and second dark patch on the posterior dorsum. In life, the female had a more reddish brown dorsal color, and reddish brown color also in the iris; it contains a large number of mature oocytes, recognizable without dissection.

Etymology. Named after the fictional character Captain Jonathan Archer, first portrayed by Scott Bakula in Rick Berman and Brannon Braga's Star Trek: Enterprise.

Tadpole. The tadpole of this species is unknown.

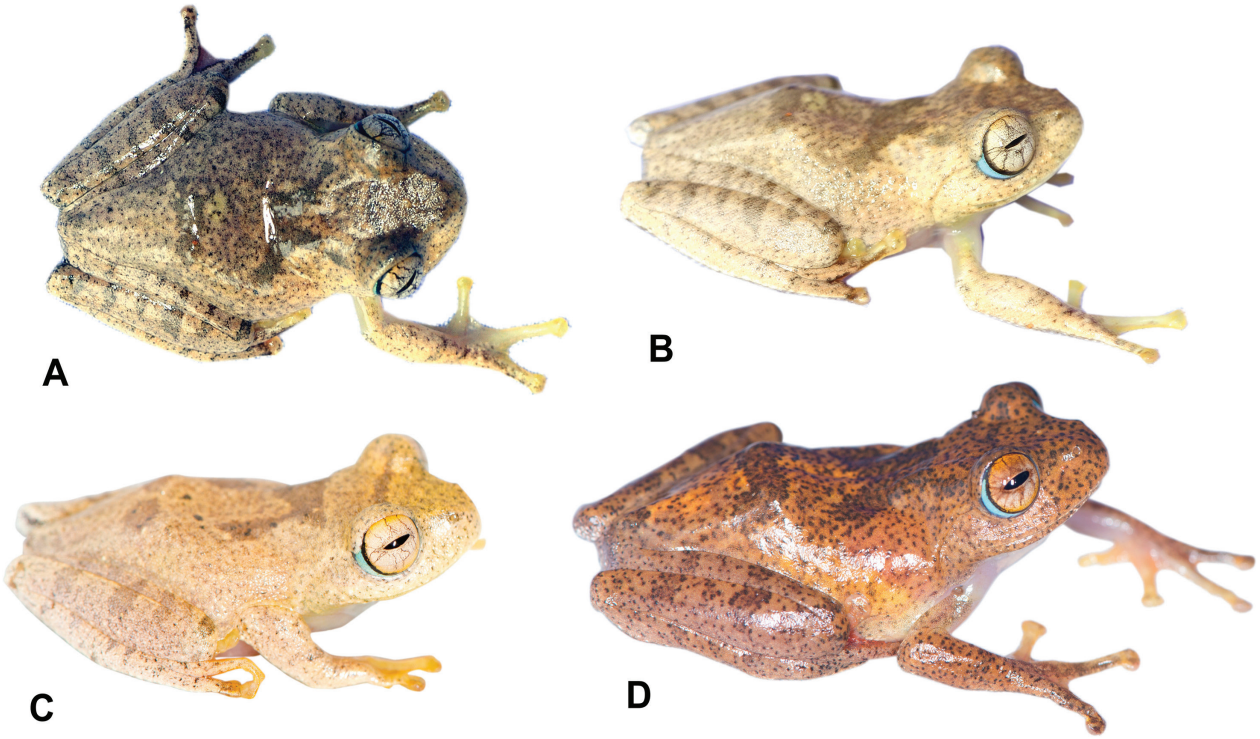

Figure 13. Individuals of *Boophis archeri* **sp. nov.** in life. **A, B** male holotype ZSM 12/2016 (MSZC 213) from Bevitagnono forest in dorsal and dorsolateral view. **C** male paratype ZSM 11/2016 (MSZC 198) from Andranonafindra forest. **D** female paratype, ZSM 13/2016 (MSZC 243) from Andranonafindra forest.

Natural history. An arboreal, nocturnal treefrog found in humid rainforests. Little is known of the ecology of the species. At the type locality, there was a remarkable density of these frogs in diminutive riparian forest fragments. Males frequently emitted calls whilst moving among the narrow twigs overhanging the stream.

Calls. Advertisement calls of *B. archeri* **sp. nov.**, recorded at Bevitagnono and Andranonafindra forests from the holotype and the male paratype on 14–15 January 2016 (air temperature not recorded), consist of multiple tonal notes, sounding like whistles. Within calls, inter-note intervals become longer from the beginning to the end of the call. All notes exhibit a distinct upward frequency modulation, with a frequency shift comprising approximately 400 Hz. The first note of each call is lower in relative amplitude when compared to subsequent notes. Amplitude modulation within notes is only slightly expressed (most distinct in first note), with increasing amplitude reaching its maximum at the second half of the note's duration. Numerical parameters of nine analyzed calls of three different individuals (among them call vouchers MSZC 198 and MSZC 213) are as follows: call duration 889–1582 ms (1242.0 ± 231.8 ms); notes/call 3–6 (4.3 ± 1.0); note duration 158–308 ms (209.3 ± 33.6 ms); inter-note interval 77–162 ms (120.7 ± 28.8 ms); dominant frequency 4130–4799 Hz (4493 ± 191 Hz); harmonic frequency band present at around 9000 Hz; prevalent bandwidth 3400–5000 Hz.

Distribution. According to molecular data summarized herein, the species is known from (1) the type locality, Bevitagnono forest, and (2) Andranonafindra forest. The known elevational range of the species spans from 1016–1169 m a.s.l.

Boophis burnhamae sp. nov.

https://zoobank.org/41140199-7BF9-416F-9FEC-52C9D92B4F66

Lineage H

Figures 6, 14

Identity. This species has been previously referred to as *B.* sp. 26 by Vieites et al. (2009), *B. marojezensis* [Ca26] by Randrianiaina et al. (2012), and *B.* sp. Ca55 by Perl et al. (2014) and Hutter et al. (2018). Note that *B.* sp. Ca26 in Hutter et al. (2018) refers to another lineage. This species was not explicitly included or mentioned by Glaw et al. (2001) and Glaw and Vences (2007).

Holotype. ZSM 492/2014 (DRV 6295), adult male, collected on 21 June 2010 by F.M. Ratsoavina at a site locally called Andrevorevo (campsite "A") (14.3464°S, 49.1028°E, 1717 m a.s.l.), on the border of the North East and North West regions of Madagascar.

Paratypes. ZSM 491/2014 (DRV 6293), adult female with same collection data as holotype. ZSM 392/2016 (ZCMV 15171), probably a young or subadult male, collected by M.D. Scherz, A. Rakotoarison, M. Bletz, M.

Vences and J. Razafindraibe close to Camp 2 "Marojejia", Marojejy National Park (14.4348°S, 49.7660°E, 616 m a.s.l.).

Definition. A small-sized treefrog assigned to the genus *Boophis*, subgenus *Boophis*, in the family Mantellidae based on occurrence in Madagascar, presence of intercalary element between ultimate and penultimate phalanx of fingers and toes (verified by external examination), presence of webbing between fingers, presence of nuptial pads in males, and absence of femoral glands in males. Assigned to the *Boophis blommersae* group based on small body size (male SVL 25.0–27.2 mm), predominantly brownish dorsal coloration, absence of red color on webbing or ventral side of limbs, suctorial stream-dwelling tadpoles, and molecular phylogenetic relationships. Within the *B. blommersae* group, defined by absence of dorsolateral bands and absence of red color in outer iris area. As advertisement calls are unknown, this species can formally mainly be defined by its numerous diagnostic nucleotide positions in the mitochondrial 16S rRNA gene: MolD identified the following robust diagnostic nucleotide combination compared to all other species in the *B. marojezensis* complex (sites given relative to the full-length 16S sequence of *Mantella baroni*): "A" in the site 161, "C" in the site 179.

Diagnosis. Within the *B. blommersae* group, distinguished from *B. vittatus* by absence of dorsolateral stripes (vs. presence). Because calls of this species are unknown, a bioacoustic pairwise diagnosis with other species of the group is not possible and therefore, the species can only be distinguished from some of its close relatives (*B. archeri* **sp. nov.**, *B. blommersae*, *B. janewayae* **sp. nov.**, *B. siskoi* **sp. nov.**) by molecular diagnostic sites (see Definition above). The species can be distinguished from *B. kirki* **sp. nov.** by absence of red color in outer iris area (vs. presence in some specimens), and presence of a lateral transparent area of the integument of tadpoles (vs. absence); from *B. picardi* **sp. nov.** by larger body size (male SVL 25.0–27.2 vs. 21.3–23.2 mm), and absence of red color in outer iris area (vs. distinct in many); from *B. pikei* **sp. nov.** by larger body size (male SVL 25.0–27.2 vs. 21.4–25.0 mm); and from *B. marojezensis* by absence of rounded patches on the posterior half of the tail musculature of tadpoles (vs. presence).

Description of the holotype. Adult male, in excellent state of preservation, SVL 25.5 mm, muscle tissue removed from left thigh for molecular analysis. Body moderately slender; head slightly wider than long, of similar width as body; snout rounded in dorsal view, moderately rounded to sloping in lateral view; nostrils directed laterally, about equidistant between tip of snout and eye; canthus rostralis distinct and concave in dorsal view, loreal region slightly concave; tympanum indistinct, difficult to recognize, somewhat ovoid (higher than wide), TD 43% of ED; supratympanic fold not recognizable anteriorly and dorsally of tympanum, weakly recognizable and regularly curved posterior of tympanum; vomerine odontophores weakly developed, well-separated in two small rounded aggregations, positioned posteromedial to choanae; choanae medium-sized, rounded; maxillary teeth present. Tongue ovoid, posteriorly bifid, free. Arms slender, forearms of slightly larger diameter, subarticular tubercles single, round; metacarpal tubercles not recognizable; fingers moderately webbed and with lateral dermal fringes; webbing formula 1(traces), 2i(traces), 2e(1), 3i(2.5), 3e(1.75), 4(1.25); relative length of fingers 1<2<4<3 (finger 2 distinctly shorter than finger 4); finger discs enlarged, rounded; nuptial pads recognizable as unpigmented swelling on first finger. Hindlimbs slender; tibiotarsal articulation reaching tip of snout when hindlimb is adpressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle small, distinct, elongated; no outer metatarsal tubercle; toes broadly webbed; webbing formula 1(0.25), 2i(1), 2e(0), 3i(1.25), 3e(0.25), 4i(1.75), 4e(1.75), 5(0.5); relative length of toes 1<2<3<5<4; toe discs enlarged, rounded. Skin smooth on dorsal surfaces, throat, chest, and ventral surface of thighs, finely granular on belly; cloacal region surrounded by an area of distinct, large, white-colored granules.

In preservative, 13 years after collection (Fig. 6), dorsally light reddish brown with a moderately contrasted but distinct and complete brown hourglass marking on anterior part of the dorsum, a discontinuous broad brown transverse bar on the posterior part of the dorsum, and a narrow dark transverse bar between the eyes. Dorsum densely spotted with poorly contrasted small brown spots which partly fuse to larger markings. Limbs light brown with darker brown crossbands: about 3–5 poorly marked crossbands on forearm, 5 on shank, 7–8 on thigh. Ventrally cream, white on belly and with some dark pigment on ventral side of feet. Color of holotype in life not recorded.

Variation. The female paratype ZSM 491/2014 in preservative has a rather uniform grayish dorsal color, with a few isolated dark spots and traces of an hourglass marking, and with three pink spots on the posterior dorsum close to the cloaca. In life, paratype ZSM 392/2016 (Fig. 14) had a reddish brown color with brown hourglass marking, a beige iris with orange color in the outer iris area, especially dorsally, a relatively distinct network of brown lines, and a turquoise iris periphery.

Etymology. Named after the fictional character Captain Michael Burnham, first portrayed by Sonequa Martin-Green in Bryan Fuller and Alex Kurtman's Star Trek: Discovery.

Tadpole. The tadpole of this species (under the name *B. marojezensis* [Ca26]) was described and illustrated by Randrianiaina et al. (2012), based on the DNA barcoded specimen ZSM 1612/2007 (FGZC 2930; GenBank accession number JQ518197) from Marojejy. As typical for all tadpoles of the group, the larvae belong to the "suctorial" ecomorphological guild. They have a large oral disk used to adhere to stones in fast-flowing water, a labial tooth row formula of 7(5-7)/3, and large numbers of oral papillae (234 marginal and 430 submarginal; without dor-

Figure 14. A paratype individual (probably a young male) of *Boophis burnhamae* **sp. nov.** in life: ZSM 392/2016 (ZCMV 15171) from Marojejia campsite in the Marojejy Massif.

sal gap). They are characterized by presence of a lateral transparent area of the integument surrounding the body.

Natural history. An arboreal, nocturnal treefrog found in humid rainforests along streams. Little is known of the ecology of the species. The paratype ZSM 392/2016 was encountered at rest during the day on a leaf overhanging the path.

Calls. Unknown.

Distribution. According to molecular data summarized herein, the species is reliably known from: (1) the type locality, Andrevorevo, and (2) Marojejy at mid-elevation near Camp Marojejia. The elevational range spans between 616–1717 m a.s.l.

Discussion

The evidence presented in this study supports that the *B. marojezensis* complex consists of multiple species. Most strikingly, we found all major genetic lineages in the complex for which bioacoustic data are available (all but one) to differ in their advertisement calls, and in some cases radically so. Furthermore, we observed several cases where two or three of these lineages co-occurred in sympatry or even close syntopy (e.g., *B. kirki* **sp. nov.** and *B. picardi* **sp. nov.** at Vohidrazana), apparently without genetic admixture and under maintenance of their call differences. Although our inferences of genetic admixture are based on only few nuclear-encoded loci, we consider them to be relatively reliable in cases were multiple co-occurring individuals of each lineage were studied and they did not share alleles in any of the markers, for instance in the Marojejy Massif. At the same time, morphological differences between most of these lineages were faint and furthermore blurred by intraspecific variation. For instance, body size, which clearly differs among some of the lineages, is obviously unreliable if subadult or immature specimens are examined, and may furthermore be influenced by ecological conditions, e.g., occurrence at different elevations (Scherz et al. 2023). Iris color, which usually is constant in anuran species and often provides reliably diagnostic characters among closely related species of *Boophis* (e.g., Andreone 1993; 1996; Glaw and Vences 1997a, 1997b; Vences et al. 2005, 2010a; Glaw et al. 2010, 2018; Köhler et al. 2011), appears to show a surprising variation in lineages of the *B. marojezensis* complex as well: As illustrated in Figure 8, genotyped specimens of *B. kirki* **sp. nov.** in some cases have a strikingly red outer iris area, while other individuals have only light orange in this part of the iris, or just a uniform beige/light brown iris. Along with Glaw et al. (2021), we therefore conclude that truly morphologically cryptic species do exist in the genus *Boophis*, and in the *B. marojezensis* complex we consider all eight major genetic lineages as distinct at the species level, even if the evidence for some lineages is less strong than in others – e.g., in fully allopatric lineages where cessation of gene flow cannot be reliably assessed with the data at hand.

Despite their overall "cute treefrog" morphology, with large eyes, broad head and short snout, and enlarged finger discs, the eight species of the *B. marojezensis* complex do not stand out among Malagasy amphibians by particularly striking external structures or conspicuous colors. In contrast, the larval stages—tadpoles—of these species are highly specialized and bizarre creatures, worthy of appearance in any science fiction film. In this taxonomic revision we have not focused on these tadpoles, which were treated in-depth by Randrianiaina et al. (2012), but it is worth mentioning that the broad, papillae-rich oral discs of these larvae are used to adhere to rocks in fast-flowing streams. Even if many Malagasy frogs have stream-adapted tadpoles (Glos et al. 2022), it is remarkable that the highly specialized larvae of the *B. marojezensis* complex can be quite common, such as *B. kirki* **sp. nov.**, which Strauß et al. (2013) found in about 30% in the surveyed streams, albeit at relatively low abundances.

A further unique and striking characteristic of all species in the *B. marojezensis* complex are their male advertisement calls. Despite strong differences between several species, all of them emit high-pitched whistles that are starkly different from the archetypal frog "croak" with which the general public is generally familiar. Some of these whistles indeed are reminiscent of sounds from the Star Trek movies, such as the boatswain whistle, or Spock's original tricorder tones. Because these frogs preferably live along fast-flowing streams, their calls must stand out against a background noise of cascades and rapids. Although comprehensive studies on this topic are lacking, it is appealing to hypothesize that these whistling sounds of high spectral frequencies are particularly suited for communication in noisy stream environments (e.g., Dubois and Martens 1984; Feng et al. 2002; Penna et al. 2005; Opazo et al. 2009; Hutter 2019).

Due to the high elevational heterogeneity of eastern and northern Madagascar, streams are common in these parts of the island, and it is not surprising that representatives of the stream-breeding species of the *B. marojezensis* complex are commonly encountered in biological surveys. Although our distribution map (Fig. 4) only includes localities verified by genetic data, several import-

ant patterns are apparent. First of all, no records of the *B. marojezensis* complex exist from the rainforests in the South East, and in the southernmost confirmed location Ranomafana National Park, only a single species (*B. kirki* **sp. nov.**) appears to occur. Most species occur in northern Madagascar and five species are so far unknown from south of 16°S latitude. The 16S phylogeny presented here (Fig. 1) is only based on a short gene fragment and therefore not suitable to reliably reconstruct deep relationships among species (Chan et al. 2022), but our multi-gene tree provides a more reliable phylogenetic hypothesis. The 16S tree places *B. kirki* **sp. nov.** in a highly nested position and a clade of predominantly northern species splitting from the basal-most node, which would support a northern origin of the group. In contrast, the multi-gene tree herein (Fig. 2) as well as the previous tree published by Hutter et al. (2018) places *B. kirki* **sp. nov.** (as *B.* sp. Ca51 / lineage A) sister to all other lineages in the *B. marojezensis* complex, contradicting this hypothesis. In a wider phylogenetic context, it must be considered that the other two species of the *B. blommersae* group (*B. blommersae* and *B. vittatus*) form the sister group of the *B. marojezensis* complex (Fig. 2), and these two species also are restricted to northern Madagascar (Glaw et al. 2001; Glaw and Vences 2007; Hutter et al. 2018). We therefore currently favor the hypothesis that the *B. blommersae* group, and also the *B. marojezensis* complex, originated and underwent an initial diversification in northern Madagascar, and several species then colonized areas of the Northern Central East, with *B. kirki* **sp. nov.** boldly going beyond the rest, to reach Southern Central East (sensu Boumans et al. 2007), similar to what has been assessed for other clades of Malagasy amphibians (e.g., Kaffenberger et al. 2012: *Gephyromantis*; Rakotoarison et al. 2017: *Stumpffia*; see also Vences et al. 2009; Brown et al. 2016).

Pabijan et al. (2012) and Rodríguez et al. (2015) studied predictors of the amount of genetic divergence and phylogeographic structure in tropical frogs and used a series of frog species supposedly occurring in both the Andasibe and Ranomafana regions (Northern Central East and Southern Central East of Madagascar according to Boumans et al. 2007) in their analysis. Recent research has shown that some of the genetically divergent populations in fact are differentiated at the species level (e.g., the *Boophis boehmei* species complex, see Vences et al. 2010b; the *Guibemantis liber* species complex, see Koppetsch et al. 2023), and such is the case with *B. kirki* **sp. nov.** and *B. picardi* **sp. nov.** described herein. While this does not invalidate the conclusions of Pabijan et al. (2012) and Rodríguez et al. (2015), the in-depth analyses are showing a pattern more complex than a strict allopatric distribution of the Andasibe vs. Ranomafana lineages. For instance, Glaw et al. (2010) found for the supposedly allopatric species pair *B. goudoti* (Andasibe region) and *B. obscurus* (Ranomafana region), that one individual of the *B. obscurus* lineage had a mitochondrial sequence of *B. goudoti*, which may represent mitochondrial introgression or co-occurrence of the two species in Ranomafana. As a second example, Glaw et al. (2021) found that a supposed pair of allopatric sibling species called *B. sandrae* and *B.* cf. *sandrae* by Glaw et al. (2010), in fact are a trio of species of which one, *B.* sp. Ca37, apparently occurs both in Andasibe and Ranomafana. For the *B. marojezensis* complex, the study of Pabijan et al. (2012) is based on a comparison of populations that we here named *B. kirki* **sp. nov.** and *B. picardi* **sp. nov.**, which are the most commonly found lineages around Ranomafana and Andasibe. However, we here also present evidence for the occurrence of *B. kirki* **sp. nov.** in syntopy with *B. picardi* **sp. nov.** in Vohidrazana near Andasibe, which adds a further species to the amazing species richness of the Andasibe region (e.g., Glaw and Vences 2007; Vieites et al. 2009; Gabriel et al. 2024). This also illustrates that even in well-studied areas of Madagascar, intensive fieldwork can yield observations of locally rare species that challenge current biogeographic knowledge.

The habitats of the *Boophis marojezensis* complex are threatened and fragmented, and conservation efforts are needed to increase the protection of the remaining natural vegetation. This is in particular of relevance for several apparently microendemic species of northern Madagascar, such as *B. archeri* **sp. nov.**, *B. siskoi* **sp. nov.**, and *B. burnhamae* **sp. nov.**, for which a categorization in one of the threatened categories of the International Union for the Conservation of Nature's Red List (IUCN 2023) should certainly apply. Other species such as *B. kirki* **sp. nov.**, *B. picardi* **sp. nov.**, *B. pikei* **sp. nov.**, *B. janewayae* **sp. nov.** and *B. marojezensis* occur each in at least one of Madagascar's National Parks or Special Reserves, and have an overall larger extent of occurrence, and therefore may still be relatively safe from immediate extinction. However, given the ongoing deforestation in Madagascar, there is no doubt that immediate conservation actions are needed, and should also include the conservation of small forest fragments where the last remaining populations of some microendemic species may occur. We hope that the naming of seven new species after Star Trek characters, many of whom championed their own versions of nature conservation in episodes of their respective series or movies, will help to direct renewed attention to Madagascar, one of Earth's "hottest" hotspots for biodiversity conservation (Myers et al. 2000; Ganzhorn et al. 2001).

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