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A new microendemic frog species of the genus *Blommersia* (Anura: Mantellidae) from the east coast of Madagascar

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Abstract

Field surveys in northeastern Madagascar have revealed the existence of a new *Blommersia* frog species (Anura: Mantellidae), populations of which were recorded within a 30 km radius of the town of Maroantsetra. We combined morphological, bioacoustic and molecular techniques and show that it is indeed a distinct evolutionary lineage which we describe as *Blommersia variabilis* **sp. nov.** from the type locality Ambodivoahangy near Maroantsetra. This new species is morphologically most similar to *B. wittei* by the presence of vomerine teeth and relatively small, well-delimited femoral glands, but differs from that species by advertisement call consisting of only 2–6 notes of comparatively longer duration, and a wider separation of femoral glands in males. It is associated with dense secondary vegetation fringing lentic water bodies. Anecdotal evidence suggests its life history is similar to other congeners. The restricted range of this species implies that it is microendemic, being possibly confined to the Antainambalana watershed. Mitochondrial and nuclear DNA variation show that its closest known relatives are *Blommersia galani* and *B. dejongi*, both of which also have restricted ranges on the east coast. A putative hybrid between a *B. galani* female and *B. dejongi* male with intermediate morphology was identified based on nuclear and mitochondrial DNA variation.

Key words: Amphibia, tropical biodiversity, integrative taxonomy, herpetology

Introduction

Anuran species diversity in the eastern rainforests of Madagascar rivals the amphibian species richness of other biodiversity hotspots of the Neotropics or southern Asia (Andreone *et al*. 2008). However, the inventory of Madagascar's anuran fauna is far from complete. The genus *Blommersia* (Mantellidae; Mantellinae), separated from *Mantidactylus* on account of morphological and molecular differences (Glaw & Vences 2006), harbors several candidate species in need of proper taxonomic description (Vieites *et al*. 2009). This genus encompasses small, semi-arboreal mantellid frogs that lay eggs in small clutches usually over standing water; their tadpoles drop into the water and complete development as tadpoles of a generalized type (Blommers-Schlösser 1979; Glaw & Vences 2007). These aspects of their life history contrast with those of many other mantellids which have riverine, often highly specialized tadpoles (e.g. *Mantidactylus*, *Boophis*), or are nidicolous with endotrophic development (*Gephyromantis*).

Blommersia species are restricted to Madagascar and the island of Mayotte of the archipelago of the Comoros. They are typical residents of swamps and disturbed habitats fringing primary vegetation and can be common in degraded environments such as rice fields as long as these are bordered by some remains of natural vegetation. Although the calls of these frogs are commonly heard in the more humid areas of Madagascar, individuals are difficult to locate due to their predisposition to extremely dense vegetation bordering standing water. Moreover, all

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species are externally similar, and apart from four uniquely patterned species (*B. angolafa, B. domerguei, B. grandisonae, B. kely*), only subtle morphological characters allow for unambiguous species discrimination, which is therefore very difficult in the field. *Blommersia* species thus remain relatively poorly studied, and various species still await description.

Recent advances in identifying and describing amphibian species in Madagascar have profited from large scale molecular surveys of genetic diversity in this group (Vieites *et al.* 2009) as well as intensified fieldwork. This is especially true for genera in which morphological differentiation is not immediately apparent. Divergence in mitochondrial DNA (mtDNA) in *Blommersia* suggests high levels of diversification. At present nine described species are known and at least six undescribed, genetically divergent lineages may warrant species status. These taxa fall into three main clades according to mtDNA variation (Vieites *et al*. 2009; Glaw & Vences 2006; Andreone *et al*. 2010). One of these clades includes taxa allied to *B. wittei* (at least three lineages). The second group is composed of *B. grandisonae, B. sarotra, B. kely, B. angolafa* and several unconfirmed candidate species. Both of these clades are in need of taxonomic revision. The third group includes *B. blommersae, B. domerguei, B. dejongi, B. galani,* and *Blommersia* sp. 2, the species formally described herein. Each of the three main *Blommersia* clades have broad distributions. Lineages allied with *B. wittei* range widely in the north of the island and also inhabit humid pockets of forest in the predominantly dry west. The distributions of the remaining two clades parallel Madagascar's north-south axis of mid-elevation rainforests and adjacent lowlands on the eastern seaboard. Although many regions lack comprehensive assessments of their amphibian fauna, it is apparent that at least some *Blommersia* species or lineages are microendemics restricted to a single lowland center of endemism (Vences *et al*. 2010) or some highland areas (Glaw & Vences 2002). Other species, such as *B. blommersae* or the recently described *B. angolafa*, have relatively broad distributions, but across their ranges are composed of deep intraspecific lineages that surpass the 3% divergence threshold of the mitochondrial 16S rRNA gene used to delimit candidate species in mantellid frogs (Vieites *et al*. 2009; Andreone *et al*. 2010). These observations hint at the role of isolation by distance and vicariance in structuring genetic variation and divergence within and between *Blommersia* species.

Here we add to the inventory of microendemic mantellines by formally describing a species called *Blommersia* sp. 2 in Vieites *et al*. (2009), based on an analysis of morphology, male vocalisation and molecular genetics. Herpetological surveys along the north-central east coast of Madagascar strongly suggest the range of this species is restricted to a small area in the vicinity of the Antainambalana river.

Material and methods

Blommersia sp. 2 was found opportunistically during three expeditions to the general area of Maroantsetra in north-eastern Madagascar in 1991, 2003 and 2010. Morphological and bioacoustic differences separating the Maroantsetra specimens from other known *Blommersia* were already apparent in 1991, when the species was collected within Maroantsetra and about 20 km south, near the village of Voloina. Genetic divergence of *Blommersia* sp. 2 to other congeneric taxa was established later (Vences *et al*. 2005a; Vieites *et al*. 2009; Vences *et al*. 2010). In April 2010 we collected further specimens (including the holotype) of the new species from two more localities: the vicinities of Ambodivoahangy and the village of Ambinanitelo. Both localities are located along the Antainambalana river, about 20-25 km northwest of Maroantsetra. Locality information in 2010 was recorded with GPS receivers. Frogs were collected at night using torches and head lamps. Specimens were euthanized in a chlorobutanol solution, fixed in 95% ethanol, and preserved in 70% ethanol. Specimens were deposited in the collection of Université d'Antananarivo, Département de Biologie Animale, Antananarivo (UADBA), Zoological Museum Amsterdam (ZMA), Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK), and the Zoologische Staatssammlung München (ZSM). FGMV, FGZC and ZCMV refer to F. Glaw and M. Vences field numbers. PSG refers to field numbers of P.-S. Gehring.

Morphological measurements (in millimetres) were carried out by M. Vences with a digital calliper (precision 0.01 mm) to the nearest 0.1 mm. The following abbreviations are used: SVL (snout-vent length), HW (greatest head width), HL (head length), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (nostril-nostril distance), TD (horizontal tympanum diameter), TL (tibia length), HAL (hand length), HIL (hindlimb length), FOL (foot length), FOTL (foot length including tarsus), FORL (forelimb length),

RHL (relative hindlimb length), FGL (femoral gland length), FGW (femoral gland width), and FGD (minimal distance between inner edges of femoral glands on opposite thighs). Terminology and description scheme follow Glaw & Vences (2002). Webbing formulae follows Blommers-Schlösser (1979).

Vocalizations were recorded in the field using a Tascam DR-07 digital recorder with internal microphone in 2010 and with an analogous dictaphone with internal microphone in 1991. Recordings from 2010 were saved as uncompressed files, re-sampled at 22.05 kHz and 16-bit resolution and computer-analysed using the software CoolEdit 98. Frequency information was obtained through Fast Fourier Transformation (FFT; width 1024 points). Spectrograms were obtained at Hanning window function with 256 bands resolution. Temporal measurements are given as range, with mean ± standard deviation in parentheses. Terminology in call descriptions follows Köhler (2000).

The phylogenetic relationships among known *Blommersia* lineages have been detailed elsewhere (Vieites *et al*. 2009; Andreone *et al*. 2010). Here we follow Vences *et al*. (2010) in using genetic variation to confirm the monophyly of all specimens morphologically assigned to *Blommersia* sp. 2, and their distinctiveness from its closest relatives *B. galani* and *B. dejongi*. Two molecular datasets were used for analyses: (1) a small but polymorphic fragment of the mitochondrial 16S rRNA gene (Vences *et al*. 2005a,b), and (2) a gene encoding the recombination activation protein (RAG1), a single copy nuclear marker widely used in resolving relationships among vertebrate species (Chiari *et al*. 2009). We used the *Rag1* gene to validate the relationships and test for a lack of inter-lineage gene flow between *Blommersia* sp. 2 and closely related species.

Sequences of the mitochondrial 16S rRNA gene from four specimens of *Blommersia* sp. 2 (GU984748-50, AY848104 listed as *Blommersia* aff. *wittei* MV-2005) were available from a previous study (Vences *et al*. 2010). We also incorporated all available sequences of *B. dejongi* and *B. galani* and a single representative of the more distant *B. domerguei* (AY848074), used as an outgroup. We extracted DNA from ethanol preserved tissues from four specimens (the holotype ZSM 236/2010, a paratype ZSM 237/2010, and two individuals from UADBA: FGZC 4290, FGZC 4306) of the new species collected in 2010 from the vicinities of Ambodivoahangy. We also extracted DNA from an egg clutch found in Ambinanitelo (PSG 2311). We followed the protocol of Vences *et al*. (2010) for DNA extraction, amplification of a fragment of the mitochondrial 16S rRNA gene, and sequencing.

Rag1 was amplified in a total of eight specimens of the new species and multiple specimens of *B. galani* and *B. dejongi*, aiming at sampling several alleles per species. We PCR-amplified a *ca*. 630 bp fragment of the coding region of this gene using primers RAG1 FIII (TGG CAC AGG GTA TGA TGA RA) and RAG1 RIII (TCA ATG ATC TCT GGG ACG TG) (primers developed by A. Crottini). PCR conditions were as follows: initial denaturation at 94°C (120 sec.) and then 38 cycles of denaturation at 94°C (20 sec.), primer annealing at 53°C (45 sec.) and elongation at 72°C (120 sec.), followed by a final extension step at 72°C (10 min.). Following Exonuclease I and Shrimp Alkaline Phosphatase digestion, the PCR products were sequenced in both directions with the PCR primers using BigDye v3.1 cycle sequencing chemistry and run on a 3130xl genetic analyzer (Applied Biosystems).

Sequences were edited and aligned in CodonCode Aligner v3.0.3 and deposited in GenBank under accession numbers JF314327–JF314331 (16S rRNA) and JF314332–JF314354 (*Rag1*). Basic analyses of sequence variation were performed in Mega 4.0 (Tamura *et al*. 2007). Several heterozygous nucleotide sites were detected in the *Rag1* dataset, therefore we used the software Phase v2.1.1 (Stephens *et al*. 2001; Stephens & Scheet 2005) to infer haplotypes (three runs under different starting seed numbers with 1000, 5,000 and 10,000 iterations). We used haplotype reconstructions from the run with the highest average goodness of fit to the underlying coalescent model (no recombination, 10,000 iterations, other parameters set at default values).

Molecular phylogenetic relationships based on the 16S rRNA data were inferred by maximum parsimony and two model based analyses. For the maximum parsimony analysis, a heuristic search with 100 random taxon stepwise addition sequences and tree bisection reconnection (TBR) branch-swapping was conducted in PAUP 4.0b10 (Swofford 2003). Nucleotide sites were coded as unordered and equally weighted characters. The topology was reconstructed using the 50% majority rule consensus with support values assessed by 1,000 bootstrap pseudoreplicates. Next we applied the Akaike information criterion in MrModelTest v2.3 (Posada & Crandall 1998; Nylander 2004) and selected the GTR + G model as the best fit model of nucleotide substitution, which was implemented in further analyses. A Bayesian analysis in MrBayes v3.1.2 consisted of two runs with four chains run for a total of 10 million generations, sampled every 100th generation. The two runs had achieved stationarity after the first 10,000

generations (discarded as burnin) as judged by plotting the generation numbers against their log-likelihoods, leaving 90,000 trees from which a majority rule consensus was produced. Support for the resulting phylogram was assessed by examining the frequency of samples recovering each clade (Huelsenbeck & Ronquist 2001). A maximum likelihood analysis was run in PhyML (Guindon & Gascuel 2003) with parameters set to the GTR model. Support for the resulting topology was obtained by bootstrapping with 1,000 replicates. The relationships of *Rag1* haplotypes within and between the four studied *Blommersia* species were examined in a statistical parsimony framework with a 95% cut-off in the software TCS v1.21 (Clement *et al*. 2000).

Results

Molecular phylogenetics

The 16S rRNA tree depicted in Fig. 1 confirms the identity of the specimens sampled in 2010 and reaffirms the genetic distinctiveness of *Blommersia* sp. 2. Out of the 333 bp analysed in the 16S rRNA gene (we shortened the original ca. 500 bp alignment to accommodate some of the GenBank sequences), 55 nucleotide sites were variable, out of which 46 were parsimony-informative. On average, there were 29.6 ± 4.9 nucleotide substitutions between *Blommersia* sp. 2 and *B. galani*. A similar value, 30.1 ± 4.9, separates *Blommersia* sp. 2 and *B. dejongi*. This number of nucleotide substitutions results in an 8.9–9.0% uncorrected divergence between the new species and its closest known relatives. Considerably more substitutions, 37.1 ± 5.6 (11.1% p-distance), occur between *Blommersia* sp. 2 and *B. domerguei*. There were 7 segregating nucleotide sites within the new species, giving an average of 3.2 ± 1.2 nucleotide differences between individuals.

We aligned a total of 612 nucleotide sites of the *Rag1* gene, corresponding to amino acid positions 731–933 of the complete *Silurana tropicalis* RAG1 protein (NCBI Reference Sequence: NP 001165554). There were 22 parsimony informative sites out of a total of 28 variable nucleotide positions in the *Rag1* alignment including *Blommersia* sp. 2, *B. dejongi* and *B. galani* (31 variable sites if *B. domerguei* was included, Fig. 3). On average, approximately the same number of nucleotide differences separated *Blommersia* sp. 2 from *B. dejongi* and *B. galani*: 12.4 ± 2.8 and 12.3 ± 3.0, respectively (2.0% p-distance). Divergence in nucleotides between *B. dejongi* and *B. galani* is lower and amounts to 8.2 ± 2.5 substitutions (1.3% p-distance). All three species could be clearly separated in the haplotype network (Fig. 2).

Heterozygous sites in the *Rag1* dataset were frequent (Fig. 3). In *Blommersia* sp. 2, we encountered 4 homozygous individuals, one individual with 4 heterozygous sites and 3 individuals with 9 heterozygous sites. In *B. dejongi*, only a single individual was a homozygote, all others possessed between 1 and 3 heterozygous sites. All *B. galani* specimens were homozygotes, with the exception of a putative hybrid (see below). Examination of the results of computational phasing revealed that not all haplotype pairs could be scored with high confidence. First, the outgroup, *B. domerguei*, possessed four heterozygous sites, three of which were also polymorphic in our ingroup species (Fig. 3). We therefore decided to remove this sequence, and assessed the relationships between the ingroup species by using a statistical parsimony network instead of a phylogenetic tree. Probabilities associated with correct inference of haplotype pairs were also low (0.413–0.612) in one specimen of the new species (ZCMV 802) and three *B. dejongi* specimens (ZCMV 804, ZSM 456/2006, ZSM 459/2006). All of these cases involved individuals having 2-4 heterozygous sites from which at least one was apomorphic, i.e. the site was monomorphic in all other alleles in the dataset (Fig. 3). We decided to include these haplotypes (the pair with the highest score for each individual) in the network analysis because a wrongly inferred haplotype would only influence intraspecific relationships by slightly altering the number of haplotypes in *B. dejongi* and/or *Blommersia* sp. 2 and by changing pairwise distances between haplotypes within these species. In our case, interspecific relationships are not affected by these heterozygous sites as they generally are not parsimony informative. Moreover, several important summary statistics, such as the number of segregating sites or nucleotide diversity per site, are also independent of haplotypic information.

Within-species variation for *Rag1* was the highest in *Blommersia* sp. 2, amounting to 11 segregating sites (only one of which was a singleton site), giving an average of 3.3 ± 1.0 nucleotide differences between individuals.

Within species variation was lower in *B. dejongi*: we counted 10 segregating sites (out of which 6 were singletons) resulting in an average of 2.2 ± 1.0 nucleotide differences between individuals in this species. All *B. galani* individuals (with the obvious exception of the putative hybrid) share the same *Rag1* haplotype.

Out of the 203 amino acids, only one was polymorphic in *Blommersia* sp. 2. This was a lysine to arginine exchange (position 823 of *S. tropicalis*) in three individuals: ZSM 237/2010, FGZC 4306 and PSG 2311. We also note an amino acid substitution in a single individual of *B. dejongi* (ZSM 456/2006, proline to serine, position 825).

The *Rag1* sequence of a single individual of *B. galani* (ZSM 452/2006) apparently contains two divergent haplotypes (Fig. 3), one of which was the single haplotype of *B. galani* (H4, see Fig. 2), the other was the most common variant of *B. dejongi* (H5). This individual was previously attributed to *B. galani* by mtDNA haplotype and morphology and indeed is listed as one of the paratypes of this species (Vences *et al*. 2010). The *Rag1* sequence of this individual was confirmed by repeating the DNA extraction, PCR and sequencing. We therefore conclude that it is a putative hybrid of a *B. galani* female and *B. dejongi* male, which is credible since it was collected at a site on Nosy Boraha where these two species were found breeding in syntopy. Field notes by MV already recognized an "intermediate position of glands" in this male (see also Table 1 in Vences *et al.* 2010) suggesting that the femoral gland distance was inherited by both parents. This specimen also has traces of vomerine teeth at least on the right side and was unique among the ZSM type series of *B. galani* in showing a light middorsal line.

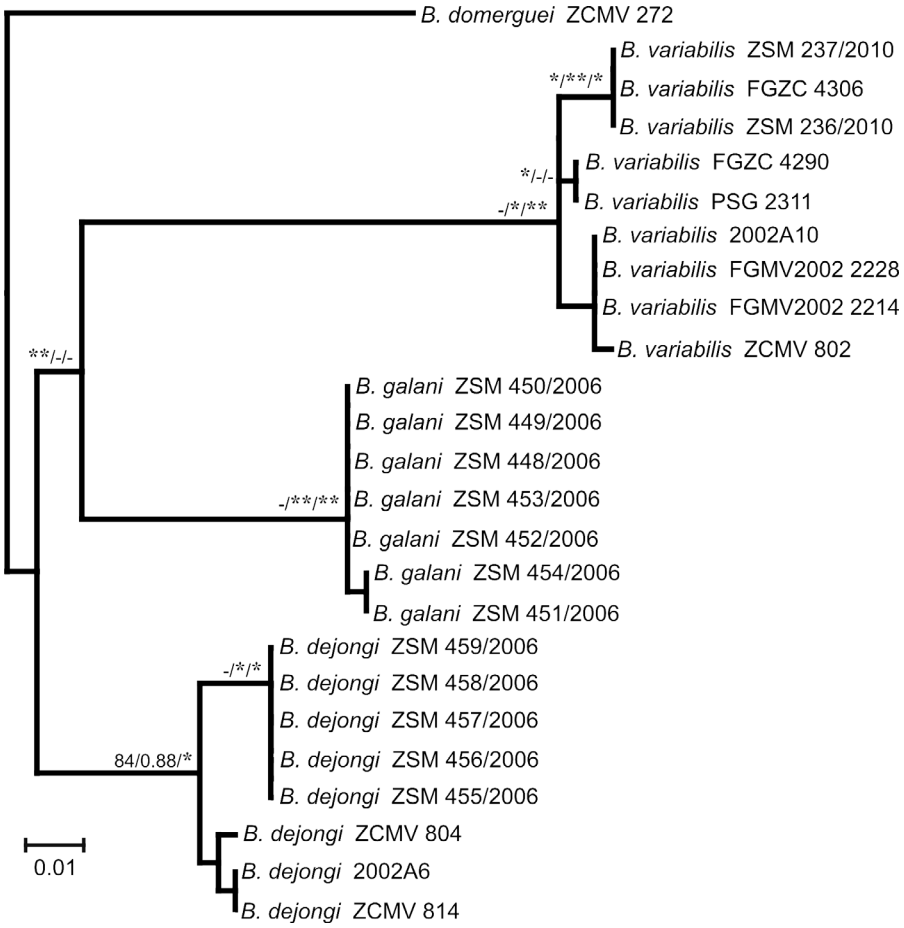

FIGURE 1. A maximum likelihood phylogram based on 16S rRNA variation depicting relationships among specimens of *Blommersia variabilis* **sp. nov.**, as well as two closely related species. Symbols at nodes indicate maximum likelihood support/Bayesian posterior probability/bootstrap support for maximum parsimony. Only values over 90% or 0.9 are shown (** – 100% or 1.00 pp; * – >90% or >0.9 pp).

Systematics

Blommersia sp. 2 can be distinguished from other *Blommersia* species by a combination of size, colouration and three morphological traits: (1) the presence or absence of vomerine teeth, (2) the extent to which lateral metatarsalia are fused or are separated only by webbing, (3) the relative position and size of the femoral glands. The presence of vomerine teeth is shared by only three species: *Blommersia* sp. 2, *B. dejongi*, and *B. wittei*. The latter two species are morphologically most similar to the new species. However, subtle differences exist in the size and shape of their femoral glands. We examined this in detail by plotting relative femoral gland length vs. relative femoral gland distance (Fig. 4) using measurements from Table 1 and information from Vences *et al.* (2010). *Blommersia* sp. 2 differs from *B. dejongi* by the placement of the femoral glands—in *B. dejongi*, these are uniquely positioned distally on the thighs next to the knee joint and thus at a much wider distance from each other. In *B. galani*, femoral glands are distinctly longer than in *Blommersia* sp. 2, and in *B. wittei*, femoral gland distance is distinctly lower than in all the other species, including *Blommersia* sp. 2. However, given the wide distribution range of *B. wittei* and the existence of two confirmed candidate species related to *B. wittei*, a more extensive assessment of its morphological variation is overdue. Regarding the connection of metatarsalia, *Blommersia* sp. 2 is characterized by remarkable intraspecific variability (see Variation below) but most specimens have partly connected metatarsalia, which constitutes a difference to *B. wittei* which has unconnected metatarsalia. Furthermore, *Blommersia* sp. 2 differs from all other *Blommersia* species by a unique male advertisement call as described below.

The concordance between morphological, bioacoustic and molecular datasets leads us to conclude that *Blommersia* sp. 2 represents a true species under the evolutionary species concept. We therefore scientifically name this species and provide a detailed description of its morphology, call and distribution.

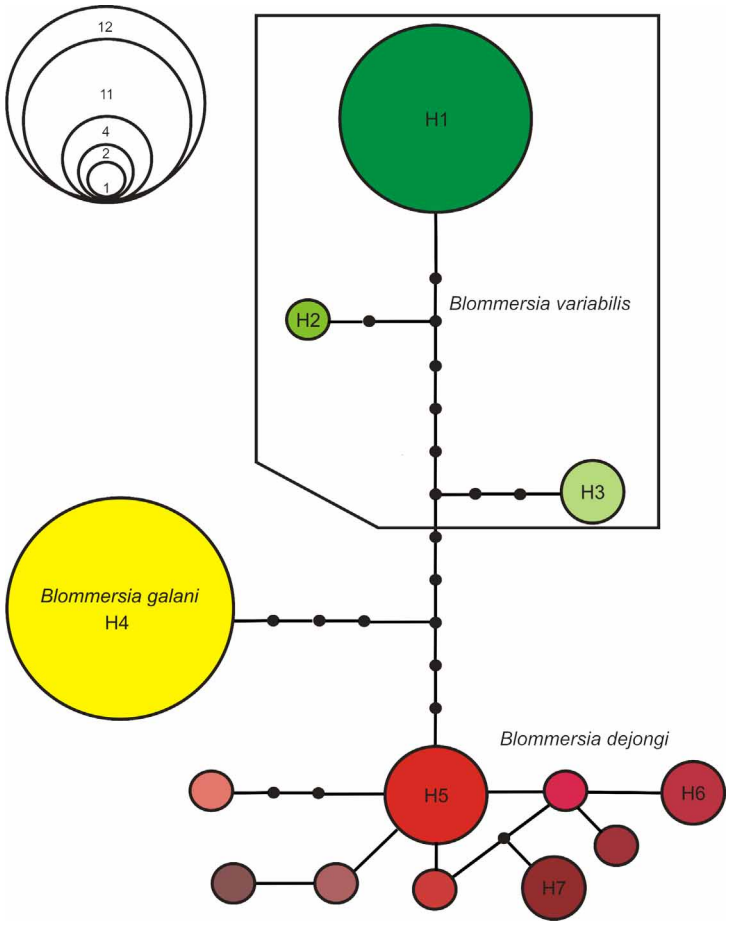

FIGURE 2. Haplotype network based on variation in *Rag1* sequences in three microendemic *Blommersia* species from the east coast of Madagascar. Haplotypes inferred by Phase v2.1.1 software (Stephens *et al.* 2001; Stephens & Scheet 2005). H1-H7 denote major haplotypes.

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B. domerguei ZCMV 272          TTCTTCYTCACAGYATAACWRGTCTGAGCCA
B. variabilis ZSM 237/2010     ACYA·TC···Y·RC·WMR·TGRYY·····G
B. variabilis FGZC 4306        ACYA·TC···Y·RC·WMR·TGRYY·····G
B. variabilis PSG 2311         ACYA·TC···Y·RC·WMR·TGRYY·····G
B. variabilis ZSM 236/2010     ACTA·TC···T··C·AC··TG·CT·····G
B. variabilis FGZC 4290        ACTA·TC···T··C·AC··TG·CT·····G
B. variabilis ZCMV 802         ACTA·TC···TR·C·AC··TG·YY·····R
B. variabilis FGMV2002.2214    ACTA·TC···T··C·AC··TG·CT·····G
B. variabilis FGMV2002.2228    ACTA·TC···T··C·AC··TG·CT·····G
B. dejongi ZSM 456/2006        AC··C·C··G···C····YTG·····G·YS·
B. dejongi ZCMV 814            AC··C·C··G···Y·····TG····AG····
B. dejongi ZSM 458/2006        AC··C·C··GY··C·····TG·····G····
B. dejongi ZSM 455/2006        AC··C·C··G···C·····TG·····G····
B. dejongi ZCMV 804            AC··C·C·YGY··C·····TR·····GA···
B. dejongi ZSM 459/2006        AC··C·C··G···C·····YR·····GA···
B. dejongi ZSM 457/2006        AC··C·C··G···C·····TR·····GA···
B. galani ZSM 452/2006         AC··YYCY·R···CR····WG···Y·G····
B. galani ZSM 453/2006         AC···TCC·····CG····AG···C·G····
B. galani ZSM 448/2006         AC···TCC·····CG····AG···C·G····
B. galani ZSM 454/2006         AC···TCC·····CG····AG···C·G····
B. galani ZSM 451/2006         AC···TCC·····CG····AG···C·G····
B. galani ZSM 449/2006         AC···TCC·····CG····AG···C·G····
B. galani ZSM 450/2006         AC···TCC·····CG····AG···C·G····
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FIGURE 3. Alignment of polymorphic sites in *Rag1* sequences of *Blommersia variabilis* **sp. nov.**, *B. dejongi*, *B. galani* and *B. domerguei*. Note that one specimen of *B. galani* (ZSM 452/2006) is a putative hybrid of *B. dejongi* x *B. galani*.

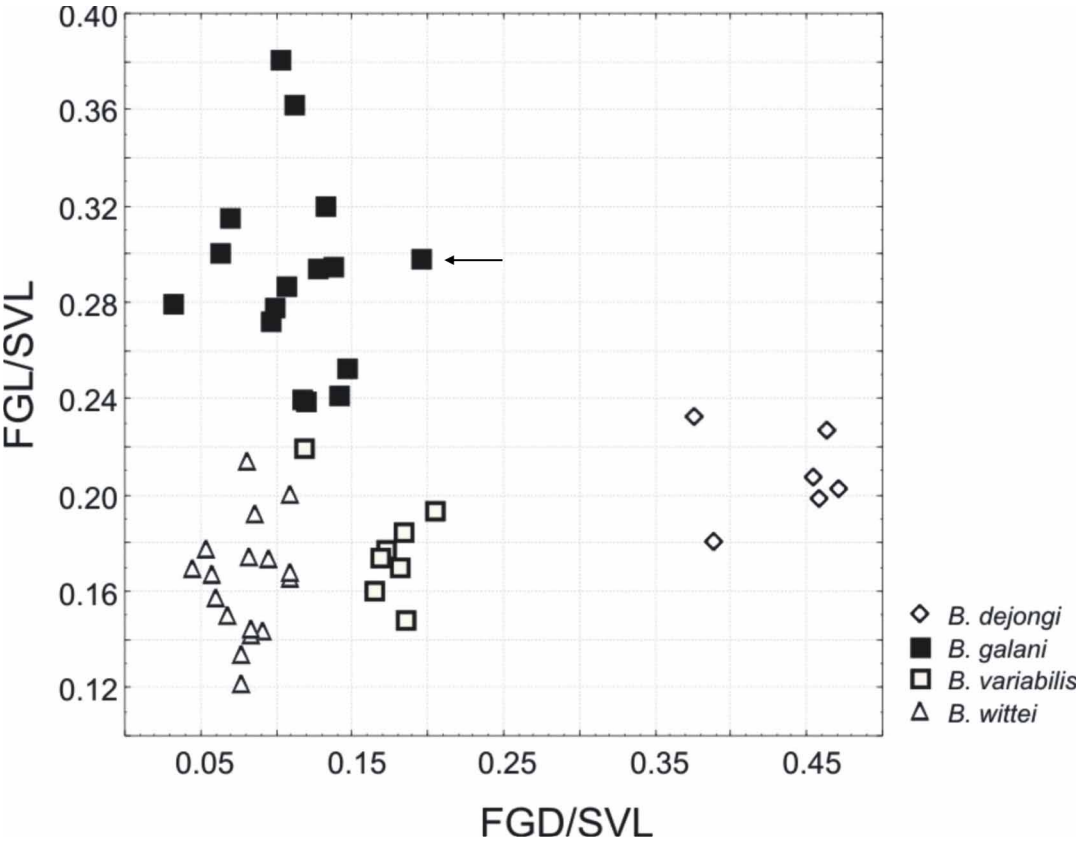

FIGURE 4. Scatterplot of relative femoral gland length (FGL/SVL ratio) and relative distance between inner edges of femoral glands (FGD/SVL) in the three endemic *Blommersia* species of Madagascar's northern central east coast, and *B. wittei*. Based on measurements provided in Table 1 (*B. variabilis* **sp. nov.** and some *B. wittei*) and Vences *et al*. (2010) for *B. dejongi* and *B. galani*, as well as type specimens of *B. wittei* (only male specimens with complete measurement data were used). Arrow points to a putative hybrid between a female *B. galani* and male *B. dejongi* (specimen ZSM 452/2006).

***Blommersia variabilis* sp. nov.**

Holotype. ZSM 237/2010 (field number FGZC 4305), adult male (Fig. 5), from Ambodivoahangy, northwest of Maroantsetra, northeastern Madagascar (coordinates: 15°17'23.8" S, 43°37'13.0" E, below 50 m a.s.l.), collected on 3 April 2010 by P.-S. Gehring, F. Glaw, J. Köhler and M. Pabijan.

Paratypes. Eleven adult males: ZSM 236/2010 (field number FGZC 4289), UADBA uncatalogued (FGZC 4290), UADBA uncatalogued (FGZC 4306), with same data as holotype; ZFMK 52608–52610, collected by F. Glaw and M. Vences on 18 March 1991 in the coastal town of Maroantsetra (15°26' S, 49°44' E, ca. 20 m a.s.l.); ZFMK 52612–52615, collected by F. Glaw and M. Vences on 19 March 1991 at a site slightly inland from the coastal village of Voloina (15°34' S, 49°37' E) which is located south of Maroantsetra; ZMA 19509 (field number FGMV 2002.2214) collected by M. Vences and A. Sarovy on 12 February 2003 in Maroantsetra.

Diagnosis. Assigned to the genus *Blommersia* in the Mantellidae by a combination of (1) presence of intercalary elements between ultimate and penultimate phalanges of fingers and toes (verified by external examination), (2) presence of femoral glands and absence of nuptial pads in males, (3) presence of a moderately distensible, inconspicuously coloured single subgular vocal sac in males, (4) small size (adult SVL < 25 mm), (5) semiarboreal habits and calling behaviour from vegetation above stagnant water. Among species of *Blommersia*, *B. variabilis* is distinguished from *B. blommersae* by vomerine teeth present (vs. absent); from *B. domerguei* by different colouration (absence of light brown dorsum with dark longitudinal markings) and vomerine teeth present (vs. absent); from *B. grandisonae* by different colouration (absence of distinct black lateral line ventrally bordered by white, vs. presence) and vomerine teeth present (vs. absent); from *B. kely* by larger size (male SVL 19–25 mm vs. 14–16 mm) and vomerine teeth present (vs. absent); from *B. sarotra* by larger size (male SVL 19–25 mm vs. 15–17 mm) and vomerine teeth present (vs. absent); from *B. angolafa* by colouration (absence of uniformly brownish dorsal colour with white dots) and vomerine teeth present (vs. absent).

The new species is morphologically most similar to *B. wittei* from which it mainly differs by the wider distance between inner margins of femoral glands (FGD 2.8–4.5 mm vs. 1.6–2.0 in the type series of *B. wittei*; see Vences *et al.* 2010; FGD 12–20% of SVL vs. 7–9%), connected metatarsalia in most specimens (vs. always separated by webbing), and advertisement calls consisting of a short series of 3-6 notes of a mean duration of 107 ms and a mean inter-note interval of 27 ms (vs. longer series of up to 25 notes with a mean duration of 23-46 ms and a mean interval duration of 30–72 ms). The new species forms a monophyletic group with the recently described *B. dejongi* and *B. galani*. It differs from *B. galani* by the presence of vomerine teeth (vs. absence) and smaller femoral glands (see Fig. 4; femoral gland length 3.5–5.2 vs. 4.8–9.2 mm). It differs from *B. dejongi* by a shorter distance between the inner margins of femoral glands (Fig. 4; FGD 2.8–4.5 mm vs. 7.8–9.8 mm). Furthermore, the new species differs from all nominal species of *Blommersia* by a substantial genetic divergence and by its advertisement call (as described below).

Description of the holotype. Specimen in a good state of preservation, tongue removed as tissue sample for molecular analysis. SVL 23.7 mm, for further measurements see Table 1. Body slender; head longer than wide, wider than body; snout rounded in dorsal and lateral views, nostrils directed laterally, slightly protuberant, nearer to tip of snout than to eye; canthus rostralis distinct, straight; loreal region slightly concave; tympanum distinct, rounded, its diameter 54% of eye diameter; supratympanic fold distinct posterior to tympanum, indistinct between eye and tympanum, curved; tongue removed, its shape therefore not verifiable; vomerine teeth present as two distinct protuberances immediately posterior-medially of choanae, maxillary teeth present; choanae small, rounded. Arms slender, subarticular tubercles single; fingers without webbing; relative length of fingers 1<2<4<3 on the right hand, 1<2=4<3 on the left hand; finger discs distinctly enlarged; nuptial pads absent. Hind limbs slender; tibiotarsal articulation reaches nostril when the hind limb is adpressed along the body; lateral metatarsalia largely separated; inner and outer metatarsal tubercles distinct; webbing formula (according to Blommers-Schlösser 1979) between toes 1(0.25), 2i(1.25), 2e(0.5), 3i(1.5), 3e(1), 4i(2.5), 4e(2.5), 5(0.75); relative length of toes 1<2<5<3<4. Skin on the upper surface smooth, without folds or ridges. No distinct enlarged tubercles in the cloacal region; ventral skin smooth. Femoral glands distinct, measuring 5.2 x 1.5 mm, of type 2 sensu Glaw *et al.* (2000), consisting of 17 distinct granules as verified from internal view (after reflexing of the ventral skin on thigh), distance between femoral glands 2.8 mm.

Table 1. Measurements of the type series of *Blommersia variabilis* **sp. nov.** and comparative specimens of *B. wittei* (all males). ZSM 237/2010 is the holotype, all other *B. variabilis* specimens are paratypes. For abbreviations, see Material and Methods. Relative hindlimb length (RHL) is coded as follows: when hindlimb is adpressed along body, tibiotarsal articulation reaches (1) anterior eye corner, (2) eye center, (3) between eye and nostril, (4) nostril, (5) snout tip.

Locality	Voucher specimen	Field number	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIBL	FGL	FGW	FGD	RHL
B. variabilis																				
Maroantsetra	ZFMK 52608	n.a.	20.6	6.6	8.3	1.5	2.6	2.2	1.4	2.3	12.3	5.9	34	15.3	9.4	10.6	3.8	1.5	3.8	4
Maroantsetra	ZFMK 52609	n.a.	18.6	6	7.8	1.4	2.4	2.1	1.6	2.4	12.4	5.5	32	14.8	9.4	10.7	3.6	1.4	3.8	5
Maroantsetra	ZFMK 52610	n.a.	20.3	6	7.9	1.4	2.3	2.5	1.4	2.1	12.2	5.7	32.6	14.7	9.8	10.6	3.6	1.5	3.5	4
Voloina	ZFMK 52612	n.a.	21.6	6.5	8.3	1.3	2.4	2.2	1.6	2.7	13.4	5.8	35.8	16.3	10	11.8	3.2	1.5	4	4
Voloina	ZFMK 52613	n.a.	24.7	6.4	9.4	1.3	2.4	2.6	1.9	2.6	14.7	7	38.3	17.3	11	12	4.2	1.6	4.5	3
Voloina	ZFMK 52614	n.a.	23.7	6.7	10	1.6	2.5	2.4	1.7	2.5	14.8	6.4	37	17	11	11.7	3.8	1.8	3.9	4
Voloina	ZFMK 52615	n.a.	21.3	6.7	8.5	1.4	2.5	2.4	1.6	2.8	12.7	6.5	37.7	16.5	10.5	11.4	3.7	1.7	3.6	4
Ambodivoahangy	ZSM 236/2010	FGZC 4289	21.4	6.3	8.2	1.5	2.5	2.1	1.5	2.1	14.2	6.6	n.a.	n.a.	n.a.	12.2	4.8	1.5	3.1	5
Ambodivoahangy	ZSM 237/2010	FGZC 4305	23.7	6.8	8.9	1.5	2.8	2.5	1.5	2.8	16	7.2	40.9	18.5	12.3	12.9	5.2	1.5	2.8	4
Maroantsetra	ZMA 19509	n.a.	22.3	6.4	8.1	1.3	2.5	2.4	1.2	2.4	13.8	6.3	34	15.8	10.3	n.a.	3.5	1.1	n.a.	1
B. wittei																				
Nosy Be	ZSM 55/2002	MV 2001.1253	24.3	7.7	9.5	2.2	2.6	2.5	1.7	2.5	15.9	7.5	41.0	19.4	13.0	n.a.	4.1	2.3	1.1	3
Nosy Be	ZSM 56/2002	MV 2001.1255	23.7	7.5	9.3	1.6	2.8	2.1	1.7	2.6	15.7	7.5	41.0	19.0	12.5	n.a.	4.2	2.0	1.3	3
Nosy Be	ZSM 57/2002	MV 2001.1265	23.0	7.2	9.0	1.6	3.0	2.2	1.5	2.4	15.3	7.2	38.4	17.7	11.9	n.a.	4.0	1.6	1.9	3
Montagne d'Ambre	ZSM 879/2003	FGMV 2002.876	22.7	7.2	8.7	1.7	2.7	2.2	2.0	2.8	15.0	7.0	35.3	16.8	11.0	n.a.	3.8	1.9	2.5	1
Montagne d'Ambre	ZSM 880/2003	FGMV 2002.878	21.8	7.0	8.3	1.5	2.3	1.9	1.6	2.6	14.2	6.7	36.3	17.2	11.3	n.a.	3.6	1.9	2.4	4
Montagne d'Ambre	ZSM 881/2003	FGMV 2002.879	21.0	6.7	8.0	1.6	2.5	2.0	1.6	2.6	13.6	6.3	34.6	15.7	10.4	n.a.	4.2	1.7	2.3	3
Montagne d'Ambre	ZSM 882/2003	FGMV 2002.880	22.0	7.0	8.3	1.5	2.5	2.0	1.6	2.3	14.1	6.6	35.3	16.5	10.7	n.a.	4.7	1.7	1.8	1
Maevatanana / Manongarivo	ZMA 19503	n.a.	20.3	6.1	8.0	1.4	2.4	2.2	1.5	2.5	13.6	6.0	36.0	16.2	10.5	n.a.	4.4	2.0	8.0	4
Nosy Be	ZMA 7160	594	24.0	7.0	9.1	1.6	2.7	2.0	1.5	2.5	16.3	6.9	40.0	19.0	12.4	n.a.	3.4	1.7	2.0	3
Nosy Be	ZMA 7160	595	25.0	7.7	9.2	1.7	2.8	2.3	1.6	2.4	16.1	7.6	41.1	19.7	13.2	n.a.	3.6	1.6	2.1	3
Ankarafantsika	ZMA 6870	1110	24.0	7.0	9.1	1.8	2.5	2.3	1.5	2.5	14.6	8.5	37.9	18.0	12.2	n.a.	4.0	1.7	1.4	2
Ankarafantsika	ZMA 6870	1108	23.1	6.9	8.6	1.5	2.8	1.9	1.4	2.1	15.1	7.2	40.4	18.4	12.7	n.a.	4.0	1.9	2.2	1
Ankarafantsika	ZMA 6870	1109	23.0	6.6	8.5	1.6	2.5	1.7	1.6	2.4	14.7	6.6	38.8	17.7	11.8	n.a.	4.4	1.5	2.0	1
Ankarafantsika	ZMA 6870	1111	23.0	6.4	8.5	1.4	2.8	2.2	1.6	2.2	15.6	6.6	38.2	17.3	11.4	n.a.	3.6	1.8	1.4	1

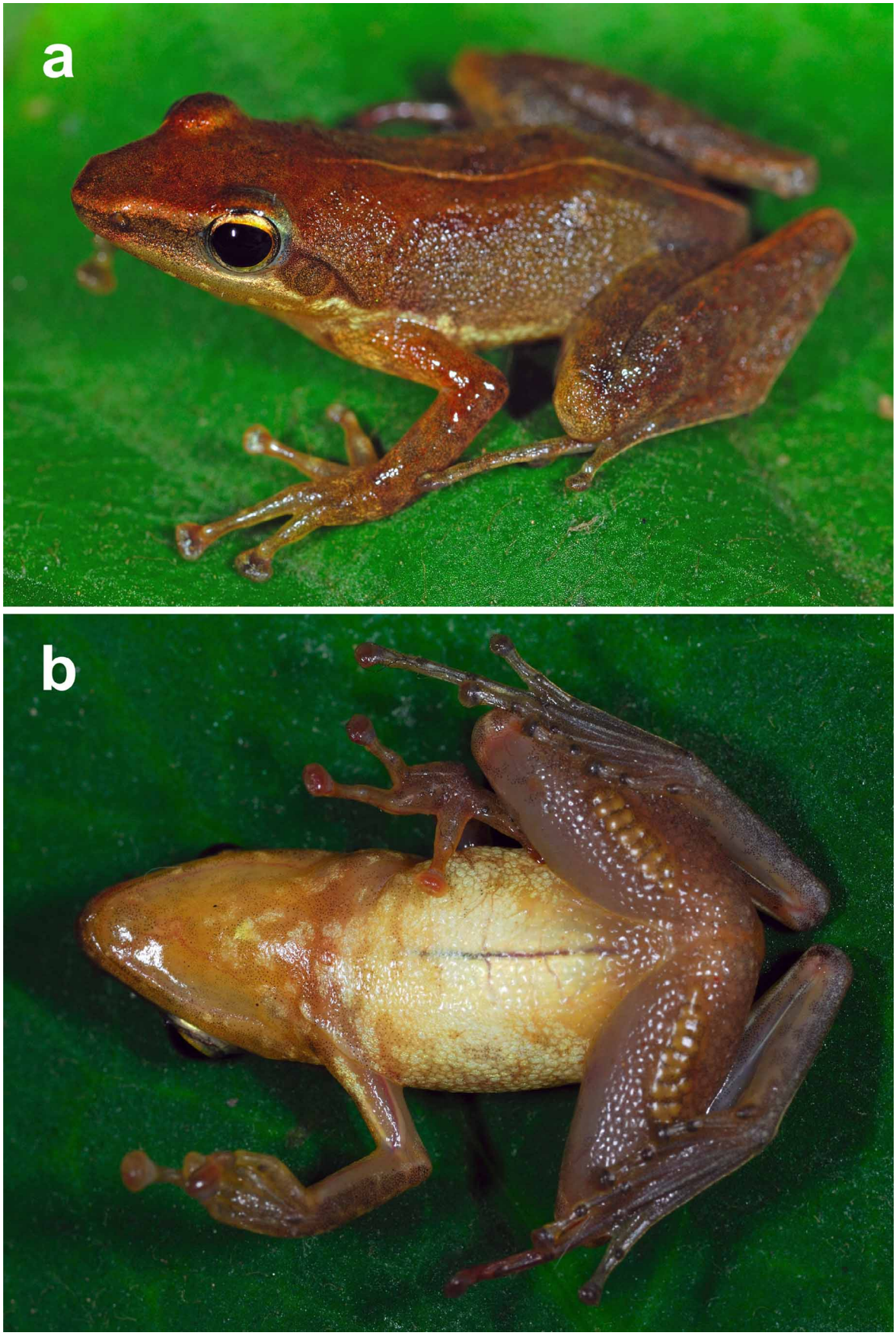

FIGURE 5. Dorsolateral (a) and ventral (b) views of the male holotype of *Blommersia variabilis* **sp. nov.** (ZSM 237/2010) in life.

After nine months in preservative, the dorsum appears greyish-brown because of many densely spaced, single dark melanophores. A translucent pink colour covers the entire dorsum, but is more prominent between and around the eye sockets and the tympanum. A thin, single medio-dorsal whitish line stretches from the neck in a posterior direction until the cloaca. There is a moderately distinct colour border between the flanks and the lighter dorsum. The hind limbs are light brown with four distinct dark brown crossbands on the thighs, three indistinct crossbands on tibia; tarsus light brown. The arms show indistinct, irregular dark patterns. Posterior to the tympanum a lateral dark streak runs along the supratympanic fold and ends at the forelimb insertion. Ventrally, the holotype was whitish with single, small and irregular dark melanophores. Throat almost uniformly whitish. Femoral glands largely white, contrasting with the yellowish colour of the inner thighs.

In life the holotype (Fig. 5) had a brown dorsum with a reddish hue on the anterior half of the body, including the head and neck, whereas the posterior is grayish-brown. Indistinct, gray crossbands line the limbs and a distinct white vertebral stripe started in the neck region and continued down the length of the body. Iris golden dorsally, ventrally poorly recognizable due to the widely opened pupil. Ventrally, the throat is yellow, but the rest of the body is white with some yellow mottling along the sternum and along the sides. The undersides of the front limbs are pinkish-blue. The ventral side of the hindlimbs is a darker blue, especially on the digits and joints. The ventral side of the thighs is pinkish-blue with yellow, clearly discernible femoral glands. The skin on the venter was translucent such that larger blood vessels could be easily discerned. The ventral colouration extends slightly onto the flanks, merging rather indistinctly with the dorsal brown colour.

Remark. This species was referred to as *Blommersia* sp. aff. *blommersae* "Maroantsetra" by Glaw & Vences (2007), *Blommersia* sp. aff. *wittei* (Maroantsetra) by Vences *et al.* (2006), and *Blommersia* sp. 2 Maroantsetra by Vieites *et al*. (2009) and Vences *et al*. (2010).

Variation. Morphological variation of nine paratypes is provided in Table 1. In general the morphology of the paratypes was similar to the holotype. However, there is some variability in the separation of the lateral metatarsalia. For instance, in ZFMK 52608 they are separated, whereas in other specimens they are partly connected, and ZFMK 52612 shows connected metatarsalia on the left foot and nearly completely separated metatarsalia on the right foot. Dorsal colouration of the paratypes is rather variable. The reddish hue characterizing the holotype is not typically observed in other specimens which are usually light brown with dark brown markings. A narrow white median line can be present on the dorsum as well as a broader band that becomes triangular at the anterior part of the dorsum, covering the entire surface of the head. Brown or grey markings and spots can be present on the venter. In 2003 we also collected one specimen of very large body size in Maroantsetra, mixed with normal-sized specimens. Because of a labeling error we cannot ascertain with full reliability the current identity of this specimen and therefore did not include it in Table 1, almost certainly it is the male ZMA 19511 of 29.0 mm SVL with largely separated outer metatarsalia (intermediate between the connected and separated state).

Etymology. The species name *variabilis* is an adjective referring to the remarkable variation observed in various characters of this new species, especially in the separation or connection of the lateral metatarsalia which otherwise is considered to be a very stable character within species of mantellids.

Natural history. At the type locality in the vicinities of Ambodivoahangy village, *B. variabilis* was common outside of the forest in cultivated landscape. It was not seen or heard within primary or selectively logged forest. Calling males were observed in dense secondary vegetation on the borders of partially inundated ricefields, about 30 cm to 1 m off the ground. The male holotype was discovered while calling within a dense bush at the edge of a rice field. During the day specimens were difficult to find, hiding underneath the leaves of plants. Calling was most frequent in the evening and at night, but was also occasionally heard during the day, especially after rains. Calls were often intermingled with choruses of *Aglyptodactylus* sp., *Guibemantis* cf. *kathrinae* and *Ptychadena mascareniensis*. At Maroantsetra it is a common species living in ditches covered by dense vegetation within the town itself. Near Voloina we heard and collected the species in flooded areas in a mosaic of rice paddies, shrubs, and remains of primary rainforest. A single clutch of eggs deposited on the surface of a leaf was discovered within the village of Ambinanitelo on 1 April 2010. Mating in this species takes place on a leaf usually overhanging lentic water and has been observed by us in Voloina. As in other *Blommersia*, the male and female sit vertically on a leaf, the male on top of the female with his legs extending over the female's dorsum (Glaw & Vences 1994).

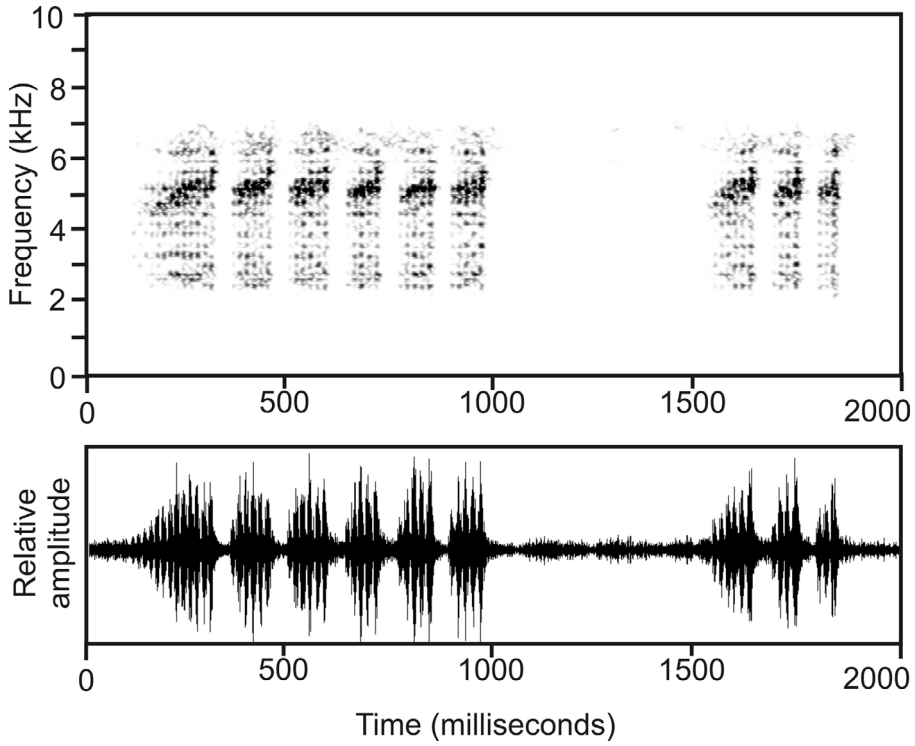

FIGURE 6. Audiospectrogram and corresponding oscillogram of the advertisement call of *Blommersia variabilis* **sp. nov.** from the type locality Ambodivoahangy, recorded on 3 April 2010.

Vocalization. The advertisement call of *B. variabilis* (Fig. 6) was recorded at the type locality (Ambodivoahangy) on 3 April 2010 at an estimated air temperature of 26°C. It consists of a series of short inharmonious notes, repeated at regular intervals in fast succession with a repetition rate of approximately 7.4 notes/second. Each call is composed of 3–6 notes, whereas in most cases a long call is followed by a shorter call containing fewer notes, in combination resulting in a call series with two calls. Within these call series, the interval between first and second call ranges between 500 and 720 ms. Within calls, the initial note is longer and contains more pulses than subsequent secondary notes. Numerical call parameters are as follows: call duration 344–887 ms (532 ± 219; n = 9); note duration 54–223 ms (107 ± 48; n = 23); pulses/note 3–14 (6.4 ± 3.0; n = 23); inter-note interval 21–33 ms (26.7 ± 3.2 ms; n = 19). Overall frequency is distributed in a broad band from app. 2000–6500 Hz, with a dominant frequency at around 5100 Hz. Calls were repeated at irregular intervals. Similar calls were recorded in 1991 in Maroantsetra, but due to the poor quality of the recordings we refrain from a detailed analysis.

Detailed call data for various populations of *B. galani*, *B. dejongi*, and *B. wittei* are presented in Vences *et al.* (2010). In comparison, the call of *B. variabilis* differs from that of *B. wittei* by longer note duration, shorter inter-note intervals and a lower note repetition rate. Calls of *B. galani* differ from those of *B. variabilis* by shorter notes and much longer inter-note intervals. In calls of *B. dejongi*, notes are barely spaced and partly fused and separate pulses within notes are unrecognizable.

Distribution. *B. variabilis* is known from (1) Maroantsetra, (2) Voloina, (3) Ambodivoahangy and (4) Ambohinantely (15°20'50.71" S, 49°35'02.84" E). The largest straight line distance between any of these sites is approximately 30 km (Ambodivoahangy-Voloina). The altitude was not precisely measured at the collecting localities, but all except Voloina are situated below 50 m a.s.l. We estimate an elevation of 100-300 m a.s.l. for the collecting locality near Voloina. The Voloina site lies close to a medium-sized river, but due to the lack of reliable coordinates for our precise collecting locality in 1991, we cannot ascertain if our samples originate from north or south of the river. The remaining three sites are in the immediate vicinity of the Antainambalana river.

Discussion

Initially, *Blommersia variabilis* was presumed to be a distinct species based on differences in morphology and male advertisement call that differentiated specimens from Voloina and Maroantsetra from *B. wittei* and *B. blommersae*. Subsequently, it became clear that this lineage is also genetically divergent from all other known *Blommersia* species (Vences *et al*. 2005a; Vieites *et al*. 2009) including other northeastern microendemics (Vences *et al*. 2010). Here we show that subtle but clear differences in morphology and male vocalisation separate *B. variabilis* from all other described *Blommersia*. Moreover, we document substantial genetic divergence between *B. variabilis* and its closest relatives in both mitochondrial (≈9%) and nuclear genes (≈2%). However, regarding the high mitochondrial divergence, it must be mentioned that this refers to a shorter fragment of the 16S rRNA gene with a higher proportion of the hypervariable regions than the fragment studied by Vences *et al*. (2005a,b), and the percentage values are therefore not directly comparable, being slightly higher than those obtained using the full fragment. Based on the integration by cumulation methodology outlined in Padial *et al*. (2010), we propose species status to this *Blommersia* lineage on account of concordance in unlinked taxonomic characters.

The relationships among *Blommersia* species were not satisfactorily resolved with the short mtDNA fragment used in this study. Previous phylogenetic results based on a much larger mtDNA fragment suggest that *B. variabilis* is the sister species of *B. galani*, which together with *B. dejongi* form a monophyletic eastern clade of lowland *Blommersia* species (Vences *et al*. 2010). Further evidence from mtDNA variation suggests that *B. blommersae* and *B. domerguei* are the closest relatives of this clade (Vieites *et al*. 2009). However, variation in the *Rag1* fragment is not entirely consistent with these results because *B. galani* and *B. dejongi* are closer to each other than they are to *B. variabilis* in the haplotype network (Fig. 2) and also in a Bayesian gene tree (not shown), although this relationship is only weakly supported. Moreover, most substitutions in the *Rag1* sequence of *B. domerguei* (mitochondrially a highly divergent species) are polymorphic in some (but not all) individuals of *B. variabilis*, *B. galani* or *B. dejongi* (Fig. 3). Clearly, the history of the clade encompassing the four *Blommersia* species studied here was more complex than suggested by the clear-cut differences in mtDNA variation. Incomplete lineage sorting, missing taxa in phylogenetic analyses and instances of past hybridization may complicate species tree inference. In *Blommersia*, the latter phenomenon is supported by the finding of a putative hybrid of *B. galani* and *B. dejongi* from the island of Nosy Boraha, where these two species occur in syntopy.

Little is known of the ecology and life history of *B. variabilis*. Calling activity has been recorded during the day and at night in March and April, at the end of the rainy season. Clutches of eggs were found attached to leaves raised slightly above the ground, over standing water. Tadpole morphology and ecology are unknown, but it is probable that the tadpoles are of generalized morphology and complete their development in water, as in all other *Blommersia* species. *B. variabilis* is apparently a common frog in the moist, tropical lowland predominated by rice cultivars and swampy areas in the environs of Maroantsetra. These habitats correspond to the vegetation categories of cultivated areas and secondary thickets (Lowry *et al*. 1997; Gautier & Goodman 2003). We have not found this species in primary or secondary forest. Syntopic species found at the type locality include *Ptychadena mascareniensis*, *Heterixalus madagascariensis*, *H. punctatus*, *Dyscophus antongilii*, *Aglyptodactylus* sp., *Boophis tephraeomystax*, *Guibemantis* cf. *liber*, *G.* cf. *kathrinae*, *Mantidactylus* (*Brygoomantis*) sp. and *Mantella ebenaui*. Although *B. variabilis* has a very small range, it seems to fare well in the anthropogenically modified landscape that now prevails in this area of Madagascar. Significant operating threats to this species were not observed. We therefore suggest a category of Least Concern according to IUCN criteria (IUCN 2001).

The range of *B. variabilis* is currently known to encompass an area extending about 30 km to the north and southwest of Maroantsetra. This species has not been found in Vatomandry, Tampolo, Foulpointe or Toamasina (Fig. 7), coastal localities at which closely related congeners occur (Gehring *et al*. 2010; Vences *et al*. 2010). In Betampona, another recently surveyed lowland locality, two described (*B. angolafa* and *B. dejongi*) and two as of yet undescribed *Blommersia* lineages are present (Andreone *et al*. 2010, A. Crottini, pers. comm.), but *B. variabilis* is apparently absent. Neither is it recorded from Befanjána (J.E. Randrianirina, unpublished) nor in mid-elevation habitats in the Makira forest (M. Vences, unpublished data). To the north, the only coastal species known so far is *B. wittei*. Therefore, it is unlikely that this species ranges widely along the eastern coast of Madagascar. It likely is a microendemic species confined to lowland localities of the northeast. Detailed fieldwork is needed to precisely

define the range limits for this species and to examine if it occurs in sympatry with other *Blommersia*. All four localities from which *B. variabilis* has been collected are close to the Antainambalana river. Wilmé *et al*. (2006) classified the area along the Antainambalana as a potential retreat-dispersion watershed (a2), embedded between an extensive lowland center of endemism (CE2). Pearson & Raxworthy (2009) dismissed the a2 retreat-dispersion watershed as ambiguous and treated this area as part of an extensive region of lowland endemism (area 2) equivalent to a combined CE2 and a2 of Wilmé *et al*. (2006). The distributions and phylogenetic relationships of *B. variabilis* and its sibling species seem congruent with the hypothesis of a lowland, coastal center of endemism in this region of eastern Madagascar, and are not indicative of a retreat-dispersion watershed. More extensive sampling of all east coast *Blommersia* species is necessary to clarify the historical centers of clade origin and speciation in this mantellid group.

Microendemicity is especially common in Madagascar's amphibian and reptile faunas (e.g. Wollenberg *et al*. 2008; Townsend *et al*. 2009). Particularly interesting are small radiations occurring only in certain geographical regions delimited by prominent features of the landscape. For example, a recently described monophyletic group of *Stumpffia* frogs inhabits the karstic areas of northern Madagascar, while another group inhabits the Marojejy massif (Köhler *et al*. 2010). A detailed focus on the distributions and phylogeny of these groups may reveal the mechanisms that have promoted species diversification leading to the rich endemic flora and fauna of Madagascar. Although vicariance scenarios are most commonly invoked, parapatric speciation along elevational gradients or even sympatric speciation are conceivable (Vences *et al*. 2009). In this context, *B. variabilis*, *B. dejongi* and *B. galani* offer an interesting example of a monophyletic, microendemic clade that is not restricted to any particular feature of the landscape, but is rather limited to the coastal lowlands of northeastern Madagascar. Large rivers flow eastward from mountainous areas in this part of Madagascar and may act as semi-permanent barriers driving divergence (e.g. Pastorini *et al*. 2003). Rivers may also fragment a previously continuous distribution and lead to vicariance, which should be especially strong along the coast, where rivers are at their widest (Vences *et al*. 2009). The role of major rivers along the east coast in the diversification of lowland *Blommersia* has yet to be tested, however, slight genetic divergence between populations of *B. variabilis* on either side of the Antainambalana river (2 substitutions in the 16S rRNA gene) suggests that even this relatively small river may obstruct gene flow in these mantellines. Divergence within lowland centers of endemism has so far been shown for lemurs (Goodman & Ganzhorn 2004; Pastorini *et al*. 2003; Pearson & Raxworthy 2009) and may have possibly played a role in day gecko diversification (Pearson & Raxworthy 2009). Our discovery of a hybrid between *B. galani* and *B. dejongi* at a site where these species clearly maintain their integrity as separate evolutionary lineages as indicated by constant differences in genes, morphology and calls, and the morphological and molecular variability of *B. variabilis*, may indicate interesting phenomena of interspecific gene flow or even hybrid speciation in these taxa, which warrant further study.

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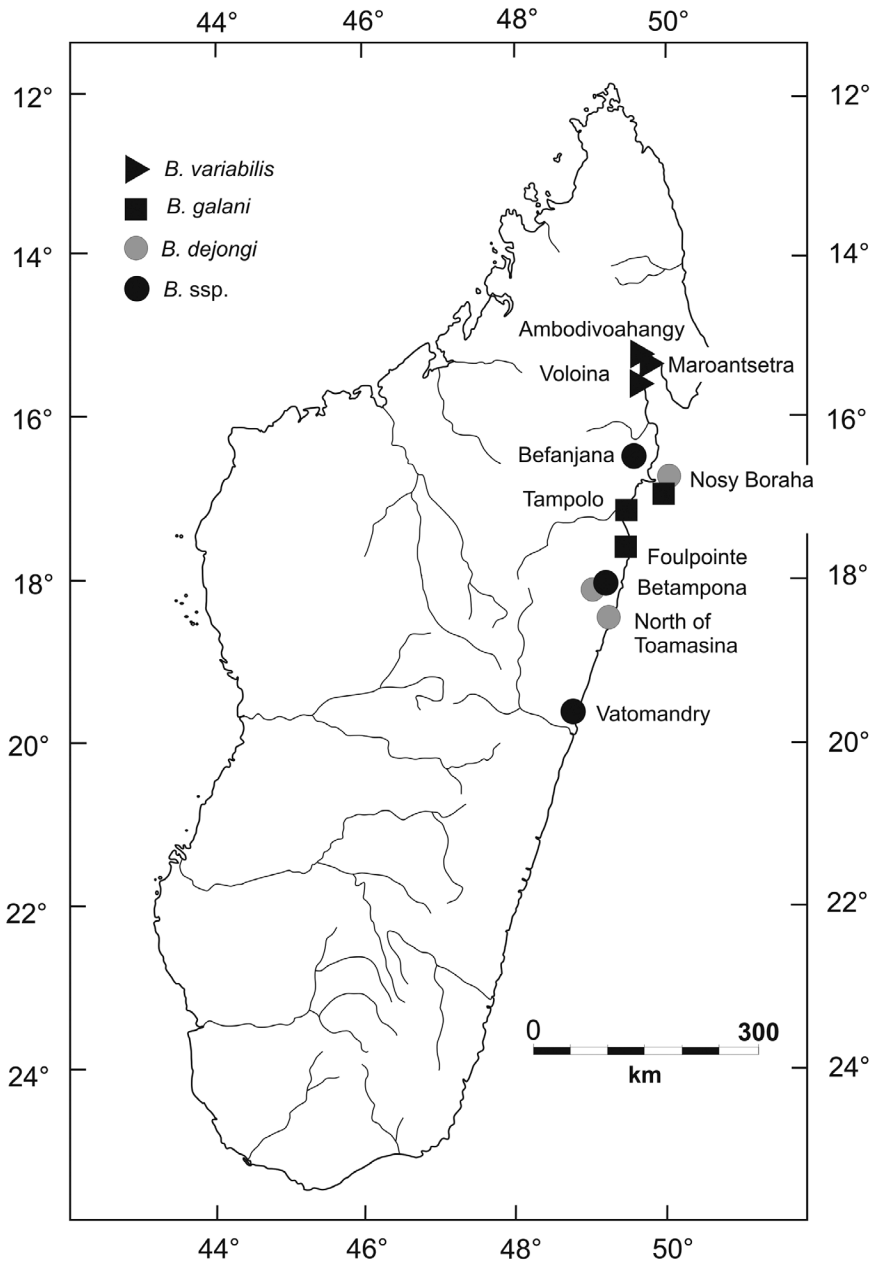

FIGURE 7. Map of Madagascar showing the east coast localities discussed in the text. Two localities only 7 km apart, Ambodivoahangy and Ambinaitelo, are depicted by a single symbol. *B.* ssp. refers to three separate and genetically divergent populations that require further study.

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