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### Two syntopic and microendemic new frogs of the genus *Blommersia* from the east coast of Madagascar

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Original article

## Two syntopic and microendemic new frogs of the genus *Blommersia* from the east coast of Madagascar

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**Abstract.**—We describe two new species of semiarborescent frogs from the northern central east coast of Madagascar which occur syntopically, at least on the island of Nosy Boraha. The two species are morphologically closest to *Blommersia wittei*, but differ in advertisement calls and molecular phylogenetic relationships. One of the new species has a remarkable femoral gland structure, as these are shifted towards the distal part of the thigh, close to the knee joint. The new species are apparently microendemic to a small stretch of Madagascar's east coast and have so far not been found at higher elevations. Since both new species are tolerant of habitat disturbance and occur in anthropogenically altered habitats, such as plantations and villages, we propose an IUCN threat status of Least Concern despite their restricted extent of occurrence.

**Key words.**—Amphibia, Anura, Mantellidae, *Blommersia galani* sp. nov., *Blommersia dejongi* sp. nov., microendemism, integrative taxonomy

Most of the world's biodiversity is concentrated in the tropics, probably because of higher rates of speciation and/or lower rates of extinction as compared to temperate biota (Moritz *et al.* 2000; Wiens & Donoghue 2004; Mittelbach *et al.* 2007; Weir & Schluter 2007). In Madagascar, one of the most important worldwide hotspots for biodiversity conservation (Myers *et al.* 2000, Kremen *et al.* 2008), this high species diversity is associated with a high degree of microendemism, with many species being restricted to very small ranges (Wilmé *et al.* 2006; Townsend *et al.* 2009; Vences *et al.* 2009; Vieites *et al.* 2009). In lemurs (Wilmé *et al.* 2006), and possibly in some Malagasy reptile groups (Pearson & Raxworthy 2009), centres of endemism appear to be related to watersheds with sources at low elevations, whereas in amphibians, e.g. in the cophyline microhylid frogs, species diversity and local endemism are related to the elevational heterogeneity of mountain massifs (Wollenberg *et al.* 2008). However, microendemism also occurs in Malagasy lowland amphibian species, such as in the poison frog *Mantella bernhardi* which has a small distribution area and, within this area, various deep conspecific lineages are restricted to even smaller ranges (Vieites *et al.* 2006). More recently, Vences *et al.* (2010) found that *Anodonthyla boulengeri*, a microhylid frog thought to be distributed along Madagascar's east coast, is in fact a complex of various distinct and unrelated species, most of which are restricted to small

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ranges. On the other hand, some Malagasy frog species are widespread without any relevant genetic differentiation, e.g. *Mantella baroni* which shares the same cytochrome *b* haplotype across a range spanning almost 700 km in latitude of mid-elevation rainforests (Rabemananjara *et al.* 2007).

Considering the high proportion of morphologically cryptic species of Malagasy frogs (e.g. Glaw *et al.* 2010), a detailed integrative taxonomic approach is necessary to precisely delimit each species and to understand the existence of possible deep intraspecific genealogical lineages before entering distributional data in a biogeographic meta-analysis. Among the many Malagasy anuran lineages containing high portions of undescribed candidate species, i.e. specimens and populations of high genetic divergences to described species and partly with divergent morphological and bioacoustic characters (Vieites *et al.* 2009), the genus *Blommersia* might be suitable for biogeographic inferences in various respects. It contains widespread species such as *B. blommersae*, *B. grandisonae* and *B. wittei*, and the phylogeography of these species can provide information on barriers to gene flow and the direction and speed of range expansions. Several populations from the central east coast of Madagascar previously assigned to *B. wittei* (Blommers-Schlösser & Blanc 1991) have long been known to be differentiated (Glaw & Vences 1994) and were recently assigned to three undescribed confirmed candidate species by Vieites *et al.* (2009). *Blommersia* are small frogs (snout-vent length ca. 15–30 mm) and within mantellines, they belong to the pond-breeding lineage and are sister to the genus *Guibemantis* (Glaw & Vences 2006; Kurabayashi *et al.* 2008). Taking into account the most recent descriptions (Glaw & Vences 2002; Andreone *et al.* 2010), the genus at present contains seven nominal species: *B. angolafa*, *B. blommersae*, *B. domerguei*, *B. grandisonae*, *B. kely*, *B. sarotra*, and *B. wittei*, as well as an additional eight undescribed confirmed candidate species. Here, we describe and scientifically name two of these candidates, based on a detailed analysis of their morphology, advertisement calls, and molecular divergences. Our data confirm the existence of microendemic amphibian species at low elevations along Madagascar's east coast, an area that so far has been insufficiently surveyed for amphibians.

## MATERIALS AND METHODS

The starting point of our study was east coast populations that previously had been assigned to *Blommersia wittei* (as *Mantidactylus wittei*) by Blommers-Schlösser (1979) and Blommers-Schlösser & Blanc (1991), but which were known to differ by advertisement calls and morphology from *B. wittei* from near its type locality Ambanja (see Glaw & Vences 1994). We examined specimens from lowland localities along the east coast of Madagascar, from an area roughly between the towns of Toamasina in the south and Maroantsetra in the north. From sites visited by ourselves (Maroantsetra, north of Toamasina, and Nosy Boraha), complete datasets on morphology, molecular genetics, and bioacoustics are available. From another site, historical material for morphological study was available (Foulpointe), and from an additional site (Tampolo), tissue samples for molecular analysis were available from the work of P.-S. Gehring. Call recordings were also available from a further site (Ile aux Nattes, next to Nosy Boraha).

Frog specimens were collected at night by opportunistic searching, using torches and head lamps. Specimens were euthanised in a chlorobutanol solution, fixed in 95%

ethanol, and preserved in 70% ethanol. Locality information was recorded with GPS receivers. 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. Additional material was examined from the Museum national d'Histoire naturelle, Paris (MNHN). Terminology for biogeographic regions of Madagascar follows Boumans *et al.* (2007).

Morphological measurements (in millimetres) were all taken by M. Vences with a digital calliper (precision 0.01 mm) to the nearest 0.1 mm. Abbreviations used are: 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 FG-FG (minimal femoral gland distance from each other). Terminology and description scheme follow Glaw & Vences (2002). Webbing formulae follow Blommers-Schlösser (1979).

Vocalisations were recorded in the field using different types of tape recorders (Sony WM-D6C, Tensai RCR-3222) and external microphones (Sennheiser Me-80, Vivanco EM 238). Recordings were 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  $\pm$  standard deviation in parentheses. Terminology in call descriptions follows Köhler (2000).

The 16S rRNA sequences of four individuals were already available from GenBank. These included two specimens from Nosy Boraha (ZSM 453/2006, GenBank accession: FJ559116; ZSM 455/2006, GenBank accession: FJ559117), one from Toamasina (2002 A6, GenBank accession: AY848077) and another from Maroantsetra (2002 A10, GenBank accession: AY848104). DNA was extracted from ethanol-preserved tissue of 17 further individuals from four different localities (Nosy Boraha, Toamasina, Maroantsetra and Tampolo). We sequenced part of the mitochondrial 16S rRNA gene using primers specifically designed to amplify a short (about 340 bp) but highly polymorphic loop region in mantellid frogs (primers 16SFrogL1 – CAT AAT CAC TTG TTC TTT AAA, and 16SFrogH1 – GAT CCA ACA TCG AGG TCG). The sequence of this fragment of the 16S gene is sufficiently variable for reliable identification of Malagasy frogs (Vences *et al.* 2005a, 2005b). In some specimens, we amplified a larger fragment (about 560 bp) encompassing the same loop region (primers 12SA-L – AAA CTG GGA TTA GAT ACC CCA CTA T, and 16SB-H, 5' – CCG GTC TGA ACT CAG ATC ACG T-3'; sequenced with 16SA-L, 5' – CGC CTG TTT ATC AAA AAC AT-3'). PCR conditions comprised initial denaturation at 94°C (90 sec) and then 36 cycles of denaturation at 94°C (45 sec), primer annealing at 51–55°C (45 sec) and elongation at 72°C (90 sec), followed by a final extension step at 72°C (5 min). Purification of the PCR products was performed by Exonuclease I and Shrimp Alkaline Phosphatase digestion. The amplicons were sequenced with either the 16SFrogL1 primer or the 16SA-L primer

(Vences *et al.* 2005b) using BigDye v3.1 cycle sequencing chemistry. Sequencing products were run on a 3130xl genetic analyser (Applied Biosystems).

Sequences were edited and aligned in CodonCode Aligner v3.0.3 and deposited in GenBank under accession numbers GU984736–GU984752. For phylogenetic analyses we incorporated three orthologous 16S rDNA sequences from closely related species as outgroups: *Blommersia wittei* (GenBank accession: AY848111), *B. cf. blommersae* (AY848059) and *B. domerguei* (AY848074). A single site was excluded from the resulting alignment due to an indel polymorphism after the addition of the outgroup sequences. Basic analyses of sequence variation were performed in Mega 4.0 (Tamura *et al.* 2007).

Phylogenetic analyses included discrete character- and model-based inference. The latter consisted of a maximum likelihood (ML) and Bayesian analyses (BI) of the nucleotide alignment after substitution model selection in MrModelTest v2.3 (Posada & Crandall 1998; Nylander 2004). Both the hierarchical likelihood ratio tests and the Akaike information criterion (AIC) implemented in MrModelTest selected GTR + G as the best fit model of nucleotide substitution. An ML phylogram was constructed 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, as implemented in the online version of PhyML. The Bayesian analysis consisted of two runs with four chains run for a total of 20 million generations, and sampled every 100th tree. 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 190 000 trees from which a majority rule consensus tree was produced with posterior probabilities calculated as the frequency of samples recovering each clade (Huelsenbeck & Ronquist 2001). A maximum parsimony (MP) analysis with unordered and equally weighted characters was carried out in PAUP 4.0b10 (Swofford 2003). An heuristic search was conducted with 100 random taxon stepwise addition sequences and tree bisection reconnection (TBR) branch-swapping. The topology was reconstructed using the 50% majority rule consensus with support values assessed by 2 000 bootstrap pseudoreplicates.

We also reconstructed relationships between all described *Blommersia* species using and expanding the dataset of Andreone *et al.* (2010). We complemented the 16S rRNA sequences with fragments of cytochrome *b* (*cytb*) and cytochrome oxidase subunit 1 (COI) sequenced in *B. dejongi*, *B. galani* and *B. sp. 2* from Maroantsetra (GenBank accession numbers HM776662–HM776667) according to the protocols in Andreone *et al.* (2010). In total, three gene fragments (1326 bp) were available for 10 *Blommersia* species and five outgroups. We inferred relationships using ML in PhyML under a single GTR + I + G model as determined by AIC. The most likely topology was then bootstrapped 1 000 times to infer support. We performed a partitioned Bayesian analysis by assigning separate (and unlinked) evolutionary models for the 16S rRNA (GTR + G), *cytb* (GTR + I + G) and COI (GTR + I + G) genes as determined by AIC in MrModelTest v2.3. BI consisted of two runs with four chains run for a total of 20 million generations, sampled every 100th tree. Although convergence of the two chains was reached after 10 000 generations, we conservatively discarded the first 20 000, leaving 180 000 over which we summarised parameter values and trees. We also implemented an MP analysis in PAUP with parameters set to the same values as reported for the 16S sequences above, but with 1 000 bootstrap replicates.

## RESULTS

### Molecular Genetics

The three gene alignment (1 326 bp) contained 490 variable characters of the 16S rRNA, cytb and COI genes, of which 400 were parsimony informative. Although all three methods recovered the same topology, most nodes were only weakly supported in the ML tree ( $\ln L = -8\ 191.832$ ) and MP analysis which produced a single most parsimonious tree with a length of 1 717 steps (consistency index,  $CI = 0.400$ ; retention index,  $RI = 0.247$ ). Relationships among described *Blommersia* species were well resolved only in the partitioned Bayesian analysis in which most nodes were highly supported ( $>0.9$  posterior probability) (Fig. 1). The results are largely congruent with the analysis of Andreone *et al.* (2010) which were based on more sequence data, including nuclear genes. Importantly, *B. sp. 1* and *B. sp. 3* of Vieites *et al.* (2009), the target species of the present study, formed a monophyletic group. Also included in this clade was *B. sp. 2* from Maroantsetra as defined by Vieites *et al.* (2009), a candidate species that will not be further discussed here but will be the subject of a forthcoming paper. *B. sp. 2* was sister to *B. sp. 1*.

For the 16S dataset, a total of 333 bp positions (74 characters were variable of which 51 were parsimony informative) were available for multiple individuals of each

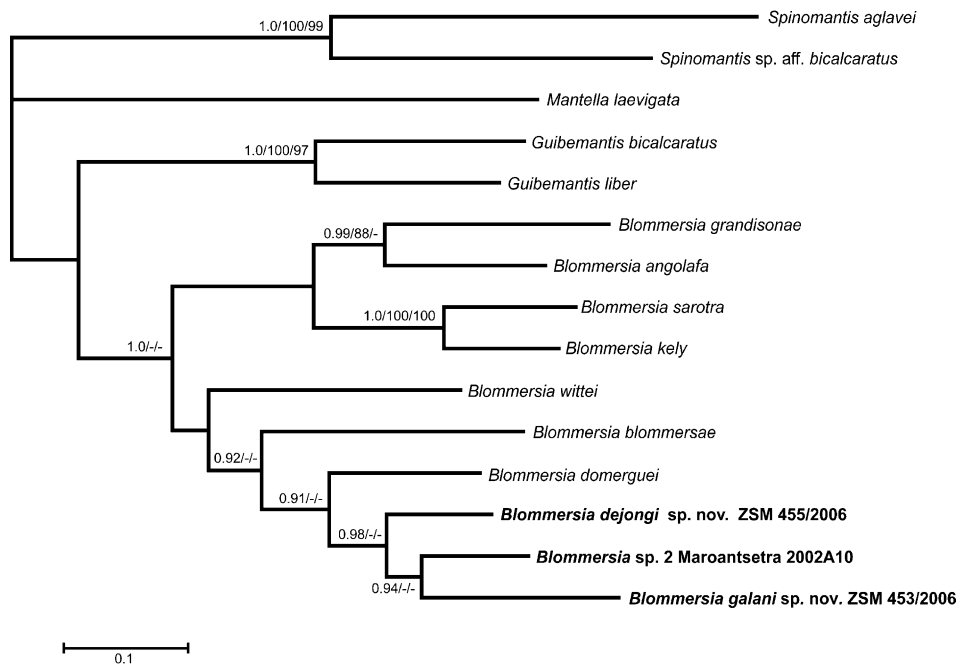


Figure 1. Phylogenetic relationships among described *Blommersia* species illustrated by a Bayesian consensus tree (mean  $\ln L = -8\ 222.15$ ) based on three mitochondrial sequence fragments (16S rRNA, cytb and COI genes) each with independent evolutionary models. Numbers next to nodes indicate posterior probabilities for Bayesian inference and bootstrap support for maximum likelihood and maximum parsimony analyses, respectively. Only values above 80% or 0.8 are shown.

of the target taxa. The three phylogenetic methods reconstructed very similar topologies for major clades, albeit with low support for basal nodes (Fig. 2). MP analysis produced two most parsimonious trees, equal with respect to all but the relationships of haplotypes within *B. sp. 2* from Maroantsetra, with a length of 115 steps (consistency index, CI=0.748; retention index, RI=0.897). The BI runs resulted in identical topologies and very similar likelihood estimates (mean  $\ln L = -1\,009.64$ ). The examined east coast *Blommersia* specimens were separated into three well supported clades, corresponding to *Blommersia* sp. 1 (in the tree already named *B. galani*, advancing the taxonomic conclusions drawn below), *B. sp. 2* from Maroantsetra, and *B. sp. 3* (*B. dejongi*). Although the three clades form a monophyletic group, this clade is retrieved with only low support by sequence variation in this fragment of the 16S gene.

On average, *Blommersia* sp. 1 and *B. sp. 3* differed by 23.1 nucleotide substitutions (6.9% uncorrected *p*-distance). *Blommersia* sp. 2 from Maroantsetra could be distinguished by 28.5 (8.6%) and 30.0 (9.0%) nucleotide differences from *B. sp. 1* and *B. sp. 3*, respectively. In contrast, sequence variation within the three clades was much lower. Haplotypes assigned to *B. sp. 1* differed by at most two substitutions

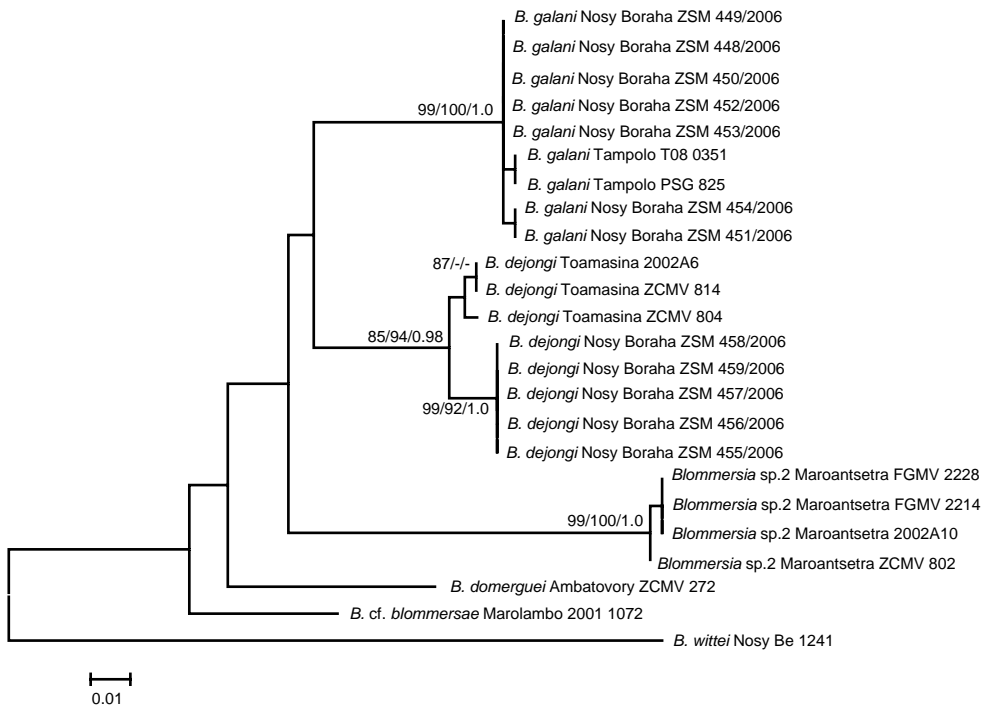


Figure 2. Phylogenetic relationships among *Blommersia* individuals from the central east coast of Madagascar depicted by a maximum likelihood phylogram ( $\ln L = -976.009$ ) based on a GTR+G model of sequence evolution. Numbers next to nodes indicate bootstrap support for maximum likelihood and maximum parsimony analyses followed by posterior probabilities for Bayesian inference. Only values above 80% or 0.8 are shown. Species names (*B. galani* = *B. sp. 1*, *B. dejongi* = *B. sp. 3*) are followed by localities and museum numbers or field numbers (if museum numbers were not available) of the examined individuals.

(0.6% uncorrected *p*-distance). *Blommersia* sp. 3 was more variable due to six nucleotide differences (1.8%) between haplotypes from the mainland population of Toamasina and the island population of Nosy Boraha. A maximum of one substitution (0.3%) separated haplotypes within *B.* sp. 2.

## Morphology

Examination of specimens from populations from the coastal stretch between Toamasina and Nosy Boraha (where molecular data identified the candidate species *Blommersia* sp. 1 and *B.* sp. 3) allowed a clear morphological characterisation of these genetically divergent species. Our analysis (original data in Table 1) first confirmed the unconnected state of the tissue surrounding the external metatarsalia which constitutes a diagnostic difference to *B. blommersae* and *B. sarotra*, two species occurring in the same geographic region but at higher elevations. Second, our analysis resulted in the identification of two clearly distinct morphological clusters among the east coast specimens. Despite a general morphological similarity and a rather high colour polymorphism, several diagnostic characters for these two clusters could be identified: one group of specimens is characterised by the lack of vomerine teeth and the presence of relatively large femoral glands in males which are almost in contact at the level of the cloaca, whereas the second group of specimens exhibits small vomerine odontophores and a unique structure of femoral glands, with their major parts located close to the knee joint. At Nosy Boraha, where specimens of both groups were found at the same site, the morphology was fully congruent with the molecular clades defined as *Blommersia* sp. 1 and *B.* sp. 3.

## Bioacoustics

Bioacoustic data were equally concordant with the morphological and molecular data. Unfortunately, despite intensive efforts to observe calling males, this could not be achieved during our 2006 survey at Nosy Boraha. However, in the swamp where specimens of both *Blommersia* sp. 1 and *B.* sp. 3 were collected, we heard two distinct calls. One of these corresponds to the call we had previously identified with full reliability from a site ca. 10 km north of Toamasina where specimens were assigned to *Blommersia* sp. 3 by molecular and morphological characters. The second call identified from Nosy Boraha in 2006 corresponds to previously recorded calls from the same locality in 1991 (see Glaw & Vences 1994) which at the time could reliably be assigned to specimens (now preserved in ZFMK; Table 1) that by morphology agree with *Blommersia* sp. 1.

## Taxonomy

Summarising the evidence from molecular, morphological and bioacoustic data, there is full concordance between these lines of evidence for the existence of two independent evolutionary lineages of *Blommersia* on the Malagasy east coast between Toamasina and Nosy Boraha, which should be considered as distinct species. Close syntopy of these two lineages at Nosy Boraha without genetic admixture provides conclusive evidence that these two lineages are reproductively

Table 1. Morphometric measurements (all in mm) of examined voucher specimens of *Blommersia galani* and *B. dejongi*, and of the morphologically similar *B. wittei*. For abbreviations of morphometric measurements and collection acronyms, see Materials and Methods. Additional abbreviations: HT, holotype; PT, paratype; M, male; F, female. RHL (relative hindlimb length) is coded as follows: when hindlimb is addressed along body, tibiotarsal articulation reaches (1) eye centre, (2) anterior eye corner, (3) between eye and nostril, (4) nostril, (5) between nostril and snout tip, (6) snout tip. The dash symbol (–) indicates that no measurement was taken or that the structure does not exist (no femoral glands in females).

| Voucher specimen         | Field number | Locality    | Type status | Sex | SVL  | HW  | HL   | TD  | ED  | END | NSD | NND | FORL | HAL | HIL  | FOTL | FOL  | FGL | FGW | FG-FG | RHL |
|--------------------------|--------------|-------------|-------------|-----|------|-----|------|-----|-----|-----|-----|-----|------|-----|------|------|------|-----|-----|-------|-----|
| <i>Blommersia wittei</i> |              |             |             |     |      |     |      |     |     |     |     |     |      |     |      |      |      |     |     |       |     |
| MNHN 1953.60             |              | Ambanja     | HT          | F   | 25.0 | 7.9 | 10.1 | 1.8 | 3.5 | 2.4 | 1.2 | 2.3 | 16.4 | 6.8 | 41.5 | 18.6 | 12.6 | –   | –   | –     | 3   |
| MNHN 1991.2529           | ex 1953.60A  | Ambanja     | PT          | F   | 23.8 | 6.2 | 9.0  | 1.6 | 3.0 | 2.5 | 1.0 | 2.2 | 13.4 | 6.5 | 36.4 | 17.4 | 12.0 | –   | –   | –     | 3   |
| MNHN 1991.2530           | ex 1953.60B  | Ambanja     | PT          | F   | 20.7 | 6.2 | 8.2  | 1.2 | 2.7 | 1.8 | 1.1 | 2.0 | 12.0 | 5.5 | –    | 15.9 | 10.6 | –   | –   | –     | –   |
| MNHN 1991.2536           | ex 1953.60H  | Ambanja     | PT          | F   | 23.0 | 6.9 | 8.7  | 1.6 | 2.6 | 2.3 | 1.4 | 2.2 | 14.4 | 5.9 | 34.7 | 16.4 | 11.4 | –   | –   | –     | 2   |
| MNHN 1991.2538           | ex 1953.60J  | Ambanja     | PT          | F   | 23.3 | 7.2 | 9.0  | 1.6 | 2.6 | 2.6 | 1.5 | 2.4 | 14.4 | 6.4 | 36.6 | 17.6 | 11.5 | –   | –   | –     | 3   |
| MNHN 1991.2531           | ex 1953.60C  | Ambanja     | PT          | M   | 21.7 | 6.6 | 8.4  | 1.4 | 2.8 | 1.7 | 1.2 | 2.2 | 14.2 | 6.1 | 33.5 | 16.2 | 10.1 | 3.1 | 1.5 | 2.0   | 1   |
| MNHN 1991.2532           | ex 1953.60D  | Ambanja     | PT          | M   | 23.4 | 7.4 | 8.5  | 1.5 | 2.6 | 2.2 | 1.4 | 2.2 | 15.0 | 7.1 | 38.0 | 17.5 | 12.0 | 3.5 | 2.0 | 1.6   | 2   |
| MNHN 1991.2533           | ex 1953.60E  | Ambanja     | PT          | M   | 21.8 | 5.7 | 8.2  | 1.5 | 2.6 | 2.0 | 1.0 | 2.1 | 15.4 | 7.0 | 38.4 | 17.9 | 11.5 | 2.9 | 1.4 | 1.7   | 4   |
| MNHN 1991.2537           | ex 1953.60I  | Ambanja     | PT          | M   | 27.0 | 8.4 | 10.6 | 1.5 | 3.2 | 2.6 | 1.5 | 2.9 | 18.5 | 8.6 | 48.0 | 22.0 | 15.2 | –   | –   | –     | 3   |
| MNHN 1991.2539           | ex 1953.60K  | Ambanja     | PT          | M   | 23.1 | 6.5 | 8.7  | 1.4 | 2.3 | 2.0 | 1.0 | 2.0 | 14.6 | 6.4 | 38.1 | 17.4 | 11.5 | 2.8 | 1.2 | 1.8   | 2   |
| <i>Blommersia galani</i> |              |             |             |     |      |     |      |     |     |     |     |     |      |     |      |      |      |     |     |       |     |
| ZSM 453/2006             | ZCMV 3232    | Nosy Boraha | HT          | M   | 21.7 | 6.8 | 8.7  | 1.9 | 2.6 | 2.3 | 1.5 | 3.0 | 13.8 | 6.3 | 37.7 | 16.7 | 11.0 | 6.4 | 2.5 | 3.0   | 4   |
| ZSM 448/2006             | ZCMV 3227    | Nosy Boraha | PT          | M   | 22.4 | 6.6 | 9.1  | 1.5 | 2.6 | 2.7 | 1.6 | 2.8 | 14.4 | 6.4 | 37.0 | 16.4 | 11.1 | 8.1 | 1.7 | 2.5   | 3   |
| ZSM 449/2006             | ZCMV 3228    | Nosy Boraha | PT          | M   | 23.2 | 6.6 | 9.3  | 1.7 | 3.0 | 2.4 | 1.6 | 2.9 | 14.7 | 6.3 | 35.6 | 16.7 | 11.0 | 5.6 | 2.3 | 3.3   | 2   |

Table 1 (Continued)

| Voucher specimen | Field number | Locality    | Type status | Sex | SVL  | HW  | HL  | TD  | ED  | END | NSD | NND | FORL | HAL | HIL  | FOTL | FOL  | FGL | FGW | FG-FG | RHL |
|------------------|--------------|-------------|-------------|-----|------|-----|-----|-----|-----|-----|-----|-----|------|-----|------|------|------|-----|-----|-------|-----|
| ZSM 450/2006     | ZCMV 3229    | Nosy Boraha | PT          | M   | 23.5 | 6.8 | 9.2 | 1.7 | 2.8 | 2.3 | 1.8 | 2.8 | 14.8 | 7.1 | 37.7 | 17.3 | 11.7 | 6.9 | 2.2 | 3.0   | 3   |
| ZSM 451/2006     | ZCMV 3230    | Nosy Boraha | PT          | M   | 23.4 | 6.8 | 9.3 | 2.2 | 2.9 | 2.4 | 1.6 | 2.9 | 14.7 | 6.9 | 36.5 | 17.2 | 11.0 | 6.7 | 2.7 | 2.5   | 3   |
| ZSM 452/2006     | ZCMV 3231    | Nosy Boraha | PT          | M   | 23.5 | 6.8 | 9.2 | 1.5 | 2.8 | 2.5 | 1.7 | 2.5 | 14.1 | 6.2 | 35.5 | 16.4 | 10.8 | 7.0 | 1.8 | 4.6   | 2   |
| ZSM 454/2006     | ZCMV 3389    | Nosy Boraha | PT          | M   | 22.5 | 6.7 | 8.7 | 1.7 | 3.0 | 2.5 | 1.5 | 2.7 | 14.4 | 6.6 | 36.4 | 16.3 | 10.4 | 7.2 | 2.5 | 3.0   | 2   |
| ZFMK 52616       | No           | Nosy Boraha | PT          | F?  | 24.3 | 6.7 | 9.1 | 1.4 | 2.9 | 2.4 | 1.6 | 2.6 | 14.5 | 6.2 | 39.0 | 18.0 | 11.6 | –   | –   | –     | 2   |
| ZFMK 52617       | No           | Nosy Boraha | PT          | M   | 21.6 | 6.7 | 8.5 | 1.6 | 2.7 | 2.5 | 1.8 | 2.7 | 13.9 | 6.8 | 37.7 | 17.0 | 11.0 | 6.8 | 2.2 | 1.5   | 4   |
| ZFMK 52618       | No           | Nosy Boraha | PT          | M   | 23.0 | 7.2 | 9.3 | 1.4 | 2.8 | 2.5 | 1.5 | 2.6 | 14.6 | 6.4 | 36.8 | 17.2 | 11.7 | –   | –   | –     | 4   |
| ZFMK 52624       | No           | Nosy Boraha | PT          | M   | 21.5 | 6.2 | 8.3 | 1.4 | 2.4 | 2.0 | 1.6 | 2.7 | 14.0 | 6.7 | 36.1 | 16.4 | 10.8 | 6.0 | 2.0 | 0.7   | 4   |
| ZFMK 52625       | No           | Nosy Boraha | PT          | M   | 23.3 | 6.8 | 8.8 | 1.4 | 2.7 | 2.2 | 1.7 | 2.8 | 14.0 | 7.0 | 37.3 | 17.2 | 11.4 | 6.2 | 1.9 | –     | 4   |
| ZFMK 52629       | No           | Nosy Boraha | PT          | M   | 24.2 | 7.6 | 9.1 | 1.5 | 2.4 | 2.4 | 1.7 | 2.6 | 15.5 | 7.0 | 42.0 | 18.5 | 12.4 | 9.2 | 2.7 | 2.5   | 4   |
| ZMA 7058         | FN 761       | Foulpointe  | PT          | M   | 20.1 | 6.0 | 7.9 | 1.3 | 2.4 | 2.0 | 1.3 | 2.0 | 12.6 | 5.6 | 33.7 | 15.4 | 10.4 | 4.8 | 1.3 | 2.4   | 3   |
| ZMA 7058         | FN 762       | Foulpointe  | PT          | M   | 20.2 | 5.9 | 8.1 | 1.4 | 2.5 | 2.0 | 1.2 | 2.3 | 13.5 | 5.8 | 32.5 | 15.0 | 8.9  | –   | –   | –     | 3   |
| ZMA 7059         | FN 604       | Foulpointe  | PT          | M   | 20.6 | 6.0 | 8.1 | 1.3 | 2.5 | 1.8 | 1.3 | 2.3 | 13.7 | 5.8 | 33.7 | 15.5 | 10.0 | 5.6 | 1.4 | 2.0   | 3   |
| ZMA 7059         | FN 605       | Foulpointe  | PT          | M   | 23.8 | 6.5 | 8.8 | 1.5 | 2.5 | 2.3 | 1.5 | 2.4 | 14.0 | 6.5 | 39.2 | 17.2 | 11.5 | 5.7 | 1.6 | 2.8   | 3   |
| ZMA 7059         | FN 619       | Foulpointe  | PT          | F   | 20.8 | 6.2 | 8.6 | 1.4 | 2.6 | 2.3 | 1.4 | 2.2 | 13.0 | 6.5 | 34.4 | 16.4 | 10.7 | –   | –   | –     | 2   |
| ZMA 7059         | FN 620       | Foulpointe  | PT          | M   | 19.0 | 5.4 | 7.9 | 1.2 | 2.2 | 2.0 | 1.5 | 2.0 | 13.0 | 5.5 | 32.3 | 14.8 | 9.5  | 4.8 | 1.5 | 2.8   | 4   |
| ZMA 7059         | FN 621       | Foulpointe  | PT          | M   | 20.2 | 6.3 | 8.4 | 1.4 | 2.4 | 2.1 | 1.4 | 2.4 | 14.5 | 6.4 | 34.0 | 15.9 | 10.6 | 5.6 | 1.4 | 2.0   | 4   |
| ZMA 7059         | FN 622       | Foulpointe  | PT          | M   | 19.0 | 6.1 | 7.7 | 1.3 | 2.3 | 1.8 | 1.3 | 2.4 | 12.7 | 5.4 | 33.4 | 14.6 | 9.1  | 5.7 | 1.9 | 1.2   | 3   |
| ZMA 7059         | FN 681       | Foulpointe  | PT          | M   | 19.4 | 5.6 | 7.9 | 1.5 | 2.3 | 1.8 | 1.3 | 2.1 | 12.1 | 5.5 | 32.0 | 14.7 | 9.6  | –   | –   | –     | 2   |
| ZMA 7061         | FN 811       | Foulpointe  | PT          | M   | 21.5 | 6.2 | 8.5 | 1.3 | 2.6 | 2.3 | 1.6 | 2.3 | 12.4 | 5.4 | 36.0 | 16.6 | 10.5 | –   | –   | –     | 3   |

Table 1 (Continued)

| Voucher specimen          | Field number    | Locality    | Type status | Sex | SVL  | HW  | HL  | TD  | ED  | END | NSD | NND | FORL | HAL | HIL  | FOTL | FOL  | FGL | FGW | FG-FG | RHL |
|---------------------------|-----------------|-------------|-------------|-----|------|-----|-----|-----|-----|-----|-----|-----|------|-----|------|------|------|-----|-----|-------|-----|
| <i>Blommersia dejongi</i> |                 |             |             |     |      |     |     |     |     |     |     |     |      |     |      |      |      |     |     |       |     |
| ZSM 455/2006              | ZCMV 3233       | Nosy Boraha | HT          | M   | 20.8 | 6.2 | 8.1 | 1.6 | 2.6 | 2.3 | 1.4 | 2.4 | 13.5 | 6.0 | 34.7 | 15.4 | 9.7  | 4.2 | 1.4 | 9.8   | 2   |
| ZSM 456/2006              | ZCMV 3239       | Nosy Boraha | PT          | M   | 19.8 | 6.2 | 7.9 | 1.4 | 2.3 | 2.0 | 1.3 | 2.0 | 13.2 | 5.7 | 33.5 | 15.3 | 10.1 | 4.1 | 1.8 | 9.0   | 3   |
| ZSM 457/2006              | ZCMV 3380       | Nosy Boraha | PT          | M   | 19.2 | 6.3 | 7.7 | 1.4 | 2.3 | 2.2 | 1.7 | 2.2 | 12.7 | 5.6 | 31.9 | 14.7 | 9.2  | 3.8 | 1.6 | 8.8   | 6   |
| ZSM 459/2006              | ZCMV 3388       | Nosy Boraha | PT          | M   | 21.1 | 6.3 | 8.2 | 1.7 | 2.5 | 2.1 | 1.6 | 2.5 | 14.3 | 6.5 | 35.6 | 16.7 | 10.8 | 3.8 | 2.1 | 8.2   | 2   |
| ZMA 19501                 | FG/MV 2002.2226 | Toamasina   | PT          | M   | 18.6 | 6.0 | 7.4 | 1.4 | 2.4 | 1.7 | 1.5 | 2.2 | 12.8 | 5.6 | 32.1 | 15.2 | 10.0 | –   | –   | –     | 3   |
| ZMA 19502                 | FG/MV 2002.2263 | Toamasina   | PT          | M   | 20.7 | 6.4 | 8.1 | 1.4 | 2.3 | 2.1 | 1.6 | 2.3 | 13.7 | 6.1 | 34.0 | 15.8 | 10.0 | 4.8 | 2.2 | 7.8   | 3   |
| ZMA 19505                 | FG/MV 2002.2266 | Toamasina   | PT          | M   | 19.4 | 6.2 | 7.4 | 1.3 | 2.5 | 2.0 | 1.3 | 2.4 | 12.4 | 5.9 | 33.3 | 15.5 | 10.2 | 4.4 | 1.6 | 9.0   | 4   |
| ZMA 19500                 | FG/MV 2002.2225 | Toamasina   | PT          | F   | 21.0 | 6.4 | 7.6 | 1.2 | 2.5 | 2.3 | 1.3 | 2.2 | 13.0 | 5.3 | 31.9 | 14.5 | 9.4  | –   | –   | –     | 1   |
| ZMA 19506                 | FG/MV 2002.2412 | Toamasina   | PT          | F   | 23.0 | 6.7 | 8.2 | 1.6 | 2.7 | 2.3 | 1.6 | 2.8 | 13.9 | 6.1 | 34.4 | 15.5 | 10.2 | –   | –   | –     | 2   |
| ZSM 458/2006              | ZCMV 3382       | Nosy Boraha | PT          | F?  | 23.6 | 6.7 | 9.0 | 1.7 | 2.7 | 2.3 | 1.8 | 2.4 | 14.5 | 6.3 | 36.6 | 16.8 | 10.7 | –   | –   | –     | 3   |

isolated and thus represent species under both the biological and evolutionary species concepts. We therefore in the following scientifically name these two species and provide a detailed description of their morphology, advertisement calls, and distribution.

*Blommersia galani* sp. nov.

**Remark.**—This species was referred to as *Mantidactylus* sp. a by Glaw & Vences (1994), *Blommersia* sp. aff. *wittei* (Nosy Boraha) by Vences *et al.* (2006), *Blommersia* sp. aff. *blommersae* ‘Nosy Boraha’ by Glaw & Vences (2007), and *Blommersia* sp. 1 by Vieites *et al.* (2009).

**Holotype.**—ZSM 453/2006 (field number ZCMV 3232, Fig. 3, right), adult male (specimen seen calling before being collected), collected at Maromandia village, 16°54.536' S, 49°52.068' E, 20 m a.s.l., Nosy Boraha island (= Ile Sainte Marie), eastern Madagascar, on 7–8 March 2006 by M. Vences & J. Randrianirina.

**Paratypes.**—ZSM 448–452/2006 and 454/2006, six adult males, same collecting locality and date as holotype; ZFMK 52616–52618, 52624–52625 and 52629, six adult males, collected at a locality on Nosy Boraha (coordinates not taken) very close to the type locality on 4–10 March 1991 by F. Glaw & M. Vences; ZMA 7058 (two specimens collected in February 1972), ZMA 7059 (seven of originally nine specimens collected on 13 February 1972) and ZMA 7061 (one specimen collected

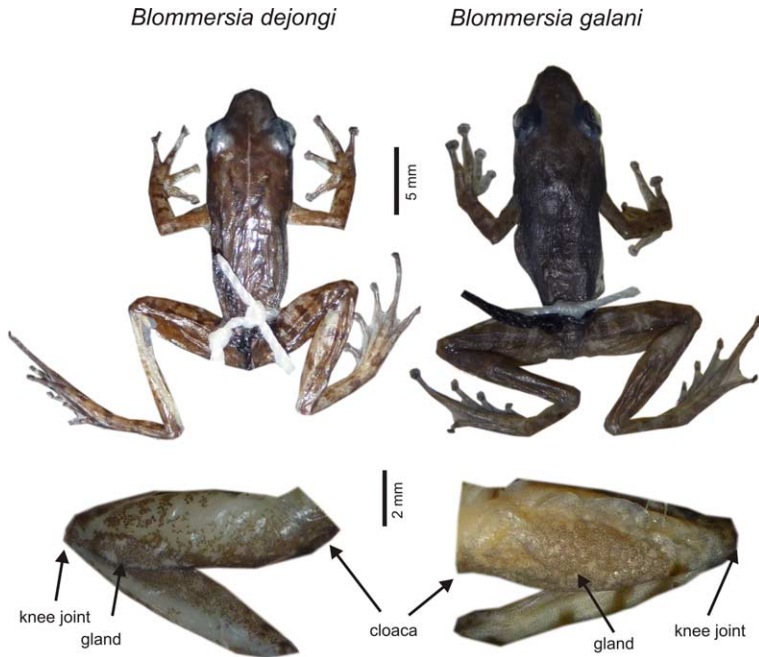


Figure 3. Preserved holotypes of *Blommersia dejongi* sp. nov. (ZSM 455/2006; left) and *Blommersia galani* sp. nov. (ZSM 453/2006; right) in dorsal view. The two lower pictures show ventral views of the thighs of the two specimens with the femoral glands. Note large gland in *B. galani* and small gland shifted close to the knee joint in *B. dejongi*.

on 6 August 1972), three series with together nine adult males and one adult female, all collected at Foulpointe by R.M.A. Blommers-Schlösser.

**Diagnosis.**—Assigned to the genus *Blommersia* in the Mantellidae by 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, not conspicuously coloured single subgular vocal sac in males; (4) small size (adult SVL <25 mm); and (5) semiarbooreal habits and calling behaviour from vegetation above stagnant water. Among species of *Blommersia*, *B. galani* is distinguished from *B. blommersae* by its lateral metatarsalia separated by webbing (vs. densely connected by tissue); from *B. domerguei* by separated lateral metatarsalia (vs. connected) and different colouration (absence of light brown dorsum with dark longitudinal markings); from *B. grandisonae* by distinctly different colouration (absence of distinct black lateral line ventrally bordered by white vs. presence); from *B. kely* by separated lateral metatarsalia (vs. connected), larger size (male SVL 19–24 mm vs. 14–16 mm); from *B. sarotra* by separated lateral metatarsalia (vs. connected) and larger size (male SVL 19–24 mm vs. 15–17 mm); from the recently described *B. angolafa* by colouration (absence of uniformly brownish dorsal colour with white dots). The new species is morphologically most similar to *B. wittei* from which it mainly differs by the larger size of femoral glands (FGL 5–8 mm vs. 3–4 mm) and the absence of vomerine teeth (vs. presence). Furthermore, the new species differs from all nominal species of *Blommersia* by a substantial genetic divergence and by its advertisement calls (as described below).

**Description of holotype.**—Specimen in good state of preservation, tissue sample removed from left thigh. SVL 21.7 mm, for further measurements see Table 1. Body slender; head longer than wide, wider than body; snout slightly pointed 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 73% of eye diameter; supratympanic fold moderately distinct, curved on left side, almost straight on right side; tongue small, ovoid, bifid and free posteriorly; vomerine teeth absent, maxillary teeth poorly recognisable; choanae small, rounded. Arms slender, subarticular tubercles single; fingers without webbing; relative length of fingers  $1 < 2 < 4 < 3$ ; finger disks distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches nostril when the hindlimb 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), 2i(1), 2e(0), 3i(1), 3e(0.5), 4i(1.5), 4e(1.5), 5(0); relative length of toes  $1 < 2 < 3 = 5 < 4$ . 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  $6.4 \times 2.5$  mm, of type 2 sensu Glaw *et al.* (2000), distance between femoral glands 3.0 mm.

After four years in preservative, the dorsum is brown with several poorly contrasting elongated darker markings on anterior regions, and a few small whitish dots posteriorly. There is a moderately distinct colour border between the darker flanks and the lighter dorsum. The hindlimbs are light brown with four distinct darker brown crossbands on thighs, three less distinct crossbands on tibia and two on tarsus. The arms also have indistinct darker crossbands. Posterior to the eye, the head

is laterally marked by a very conspicuous dark brown streak underneath the supratympanic fold, which includes the tympanum and ends close to the forelimb insertion. Ventrally dirty cream-yellowish, with fine indistinct mottling on belly and distinct mottling on thighs. Throat almost uniformly grey. Femoral glands largely brown, slightly darker than the surrounding skin.

The colour in life (Fig. 4) was similar to that in preservative, but generally lighter. The dorsum and the shanks had reddish-brown dots which have entirely been lost in preservative. The iris was golden in its upper part. Ventrally, the throat was greyish, the belly was dirty whitish with a dark median stripe shining through the skin. Ventral surfaces of limbs greyish, including the femoral glands, although their granules were more yellowish.

**Variation.**—Morphological variation of the paratypes is provided in Table 1. Dorsal colouration of all ZSM paratypes is dark brown and similar to the holotype, only ZSM 452/2006 has a thin light middorsal line which is easily recognisable from the anterior back to the cloaca. Ventral colouration is similar to the holotype as well: the throat is almost uniformly grayish-white with at most small brownish dots (in ZSM 454/2006), belly mostly with rather fine and indistinct mottling, slightly more distinct only in ZSM 451/2006 and ZSM 454/2006. Tissue samples were taken from the left shank (ZSM 448/2006, 449/2006), the right shank (ZSM 451/2006), the tongue (ZSM 452/2006) or from the right forearm (ZSM 450/2006). Additional photographs (Glaw & Vences 1994: colour photo 75; Glaw & Vences 2007: 187) show that the dorsal colouration varies from light brown to dark grey, whereas the tympanic region is distinctly darker and the flanks are at least slightly darker.

**Etymology.**—The specific name is dedicated to Pedro Galán, La Coruña, Spain, in recognition of his considerable contributions to the understanding of the Galician herpetofauna and lizard ecology, and acknowledging the enthusiastic and patient mentorship he provided to MV during his first years of herpetological interest.

**Natural history.**—The species has been found on two occasions on Nosy Boraha in flooded areas in cultivated landscape close to human settlements characterised by secondary vegetation and several trees providing partial shade for the swamps. Specimens were calling in the rainy season at night, and partly during the day, from the dense vegetation in the swamps, sitting on and often under leaves, 10–150 cm

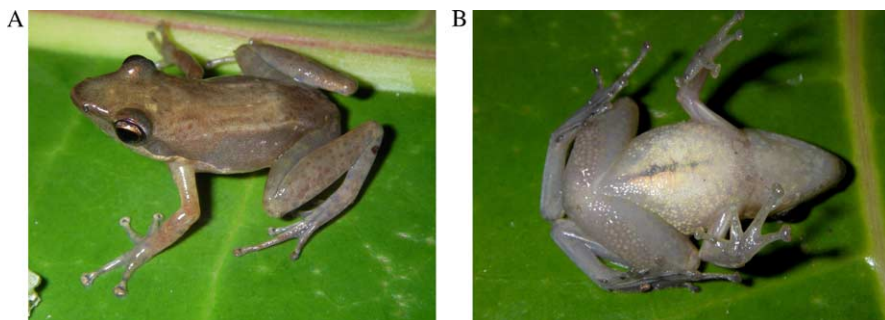


Figure 4. Male holotype of *Blommersia galani* sp. nov. (ZSM 453/2006) from Nosy Boraha in life; (A) dorsolateral and (B) ventral view.

above the shallow water. Attempts at head-clasping were observed between two males (Glaw & Vences 1994). Blommers-Schlösser (1979) reported a tadpole collected at Foulpointe that was morphologically similar to those of *B. wittei* (from Ampijoroa), but the identity of this tadpole requires confirmation.

**Vocalisation.**—The advertisement call of *Blommersia galani* recorded at the type locality (Maromandia village, Nosy Boraha) on 7–8 March 2006 at an estimated air temperature of 25°C, consists of a series of short unharmonious notes, repeated at regular intervals with a repetition rate of approximately 5–6 notes/second (Fig. 5). Each call is composed of 4–10 notes, resulting in call durations of 520–1 590 ms ( $1\,262 \pm 305$ ;  $n = 14$ ). Other numerical parameters are as follows: note duration, 25–47 ms ( $37.3 \pm 7.3$ ;  $n = 43$ ); inter-note interval, 94–210 ms ( $132.2 \pm 24.1$ ;  $n = 36$ ); dominant frequency, 5 090–5 390 Hz ( $5\,175 \pm 122$ ;  $n = 14$ ). Overall frequency is distributed in a broad band from approximately 2 000–9 000 Hz, and a fundamental frequency band with high energy is recognisable at approximately 2 300–2 880 Hz. Notes exhibit some amplitude modulation with highest energy present at the beginning, rapidly decreasing towards the end of the note. Initial notes of a series are sometimes composed of two main pulses, but are usually emitted with lower energy (Fig. 5).

Additional calls of *B. galani* from Nosy Boraha recorded in 1991 (Vences *et al.* 2006, CD 1, track 80) and those from Ile aux Nattes recorded by T. Zehrer, generally agree in characteristics with the most recent recordings and vary only slightly in temporal parameters. A common character in all calls of *B. galani* is the long duration of inter-note intervals in relation to the note duration.

**Comparative call data.**—The call of *B. galani* is similar to the calls of *B. wittei* in that both are a regular series of notes. Molecular data indicate that western populations formerly assigned to *B. wittei* belong to a separate candidate species, *Blommersia* sp. 5 (Vieites *et al.* 2009), and its calls will be described elsewhere. We here provide temporal call data for several *B. wittei* populations from the north-east, north, and Sambirano regions of Madagascar (Table 2; see also Glaw & Vences 1994). Note duration in the two species is similar, but inter-note intervals are distinctly longer and

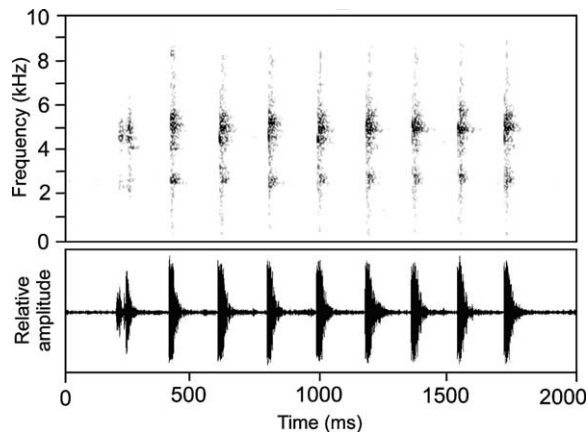


Figure 5. Spectrogram and corresponding oscillogram of the advertisement call of *Blommersia galani* sp. nov., recorded on 7–8 March 2006 at the type locality.

Table 2. Comparative temporal parameters of advertisement calls of different populations of *Blommersia wittei*, *B. galani* sp. nov. and *B. dejongi* sp. nov. Values refer to regular note series only. Data are given as range (mean  $\pm$  standard deviation and number of units analysed). NRR = note repetition rate; the dash symbol (–) indicates that no measurement was taken.

| Species           | Locality           | Note duration (ms)            | Inter-note interval duration (ms) | NRR (1/s) |
|-------------------|--------------------|-------------------------------|-----------------------------------|-----------|
| <i>B. wittei</i>  | Ambanja            | 26–68 (46 $\pm$ 12, n = 20)   | 14–48 (30 $\pm$ 12, n = 16)       | 15        |
| <i>B. wittei</i>  | Andrakata          | 18–40 (28 $\pm$ 6, n = 26)    | 62–97 (82 $\pm$ 9, n = 22)        | 10        |
| <i>B. wittei</i>  | Sambava            | 13–37 (27 $\pm$ 7, n = 23)    | 61–102 (76 $\pm$ 11, n = 17)      | 10        |
| <i>B. wittei</i>  | Sambava            | 20–28 (23 $\pm$ 2, n = 21)    | 61–78 (72 $\pm$ 4, n = 18)        | –         |
| <i>B. galani</i>  | Nosy Boraha (1991) | 14–30 (20 $\pm$ 4, n = 23)    | 128–163 (145 $\pm$ 11, n = 20)    | 8         |
| <i>B. galani</i>  | Nosy Boraha (2006) | 25–47 (37 $\pm$ 7, n = 43)    | 101–210 (132 $\pm$ 24, n = 36)    | 5–6       |
| <i>B. galani</i>  | Ile aux Nattes     | 19–46 (33 $\pm$ 9, n = 11)    | 144–188 (161 $\pm$ 19, n = 9)     | 5–6       |
| <i>B. dejongi</i> | Nosy Boraha (2006) | 72–127 (102 $\pm$ 17, n = 13) | 10–38 (27 $\pm$ 8, n = 11)        | 8         |
| <i>B. dejongi</i> | Toamasina          | 104–137 (125 $\pm$ 15, n = 7) | 19–34 (25 $\pm$ 6; n = 5)         | 6         |

note repetition rate thus is distinctly lower in calls of *B. galani*. Furthermore, the call of *B. galani* differs from those of other *Blommersia* as follows (recordings available from Vences *et al.* 2006; only the most important differences are summarised here): *B. kely* and *B. sarotra* emit single long notes, each composed of distinctly recognisable pulses (vs. fast series of short notes in *B. galani*); *B. domerguei* and *B. grandisonae* have two note types and typically one initial long note is followed by a series of secondary short notes (vs. typically only a series of short notes). *B. blommersae* typically emits a series of only 2–3 chirps of longer duration of ca. 70–150 ms (vs. 4–10 notes of < 50 ms in *B. galani*).

**Distribution.**—*Blommersia galani* is known from (1) its type locality Nosy Boraha, from (2) the southern extension of this same island, named Ile aux Nattes, based on a call recording (see Table 2), as well as from (3) Foulpointe on the opposite mainland based on morphological examination of specimens collected by R. Blommers-Schlösser (see Table 1), and from (4) Tampolo based on tissue samples provided by P.-S. Gehring and R.D. Randrianiaina included in our molecular analysis (Fig. 2). The position of these localities is shown in Fig. 6. An additional record from Fenoarivo (Glaw & Vences 2007), based on ZMA specimens collected by Blommers-Schlösser (1979) which were not available for this study, is in need of confirmation.

#### *Blommersia dejongi* sp. nov.

**Remark.**—This species was referred to as *Blommersia* sp. aff. *wittei* (Toamasina) by Vences *et al.* (2006), *Blommersia* sp. aff. *blommersae* “Toamasina” by Glaw & Vences (2007), and *Blommersia* sp. 3 by Vieites *et al.* (2009).

**Holotype.**—ZSM 455/2006 (field number ZCMV 3233, Fig. 3, left), adult male, collected at Maromandia village, 16°54.536' S, 49°52.068' E, 20 m a.s.l., Nosy Boraha

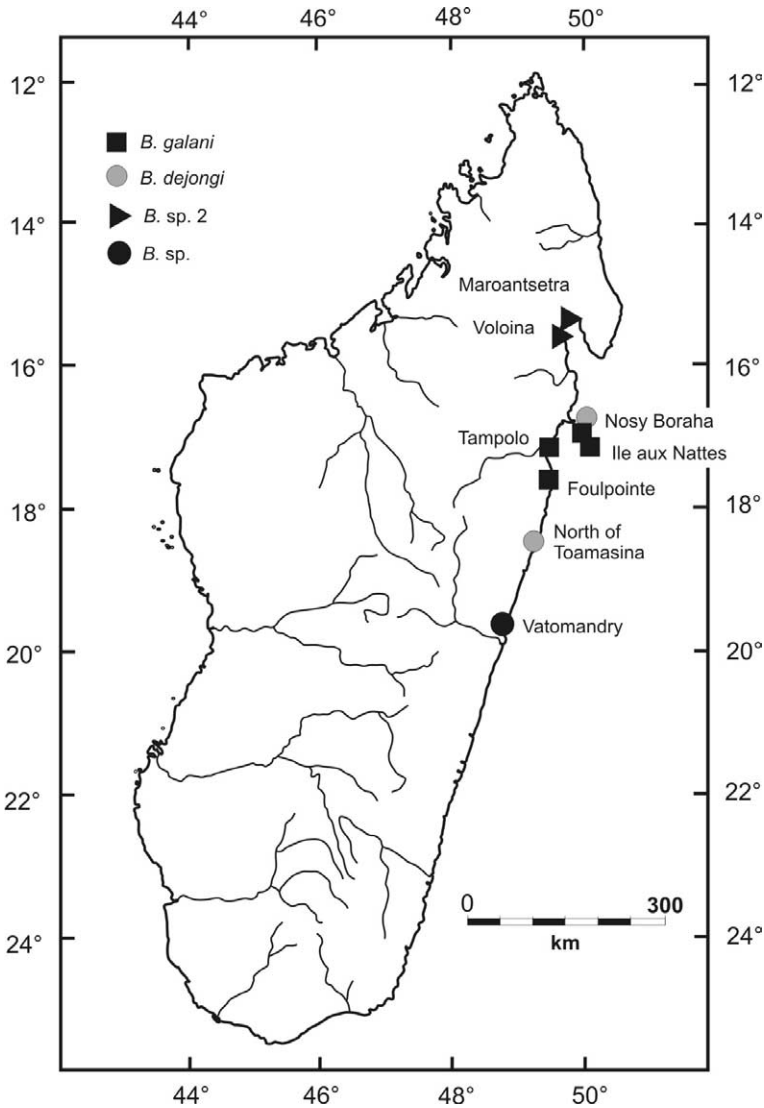


Figure 6. Map of Madagascar showing east coast localities discussed in the text.

island (= Ile Sainte Marie), eastern Madagascar, on 7–8 March 2006 by M. Vences & J. Randrianirina.

**Paratypes.**—ZSM 456–459/2006, three adult males and one adult female, same collecting locality and date as the holotype; ZMA 19501–19502 and ZMA 19505, three adult males, ZMA 19500 and 19506, two females, all collected at a locality ca. 10 km north of Toamasina along national road no. 5 (at approximately 18°02' S, 49°23.5' E, 10 m a.s.l.) on 10 February 2003 by M. Vences.

**Diagnosis.**—Assigned to the genus *Blommersia* in the Mantellidae by 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); and (5) semiarborescent habits and calling behaviour from the vegetation above stagnant water. Among species of *Blommersia*, the new species is distinguished from *B. blommersae* by its lateral metatarsalia separated by webbing (vs. densely connected by tissue) and presence of vomerine teeth (vs. absence); from *B. domerguei* by separated lateral metatarsalia (vs. connected) and different colouration (absence of light brown dorsum with dark longitudinal markings) and presence of vomerine teeth (vs. absence); from *B. grandisonae* by distinctly different colouration (absence of distinct black lateral line ventrally bordered by white vs. presence) and presence of vomerine teeth (vs. absence); from *B. kely* by separated lateral metatarsalia (vs. connected), larger size (male SVL 19–24 mm vs. 14–16 mm) and presence of vomerine teeth (vs. absence); from *B. sarotra* by separated lateral metatarsalia (vs. connected), presence of vomerine teeth (vs. absence) and larger size (male SVL 19–24 mm vs. 15–17 mm); from *B. angolafa* by colouration (absence of uniformly brownish dorsal colour with white dots); and from the syntopic *B. galani* (see above) by the presence of vomerine teeth (vs. absence). The new species is morphologically most similar to *B. wittei* but differs from this and all other species of *Blommersia* by the unique position of its femoral glands distally on the thighs next to the knee joint. Furthermore, the new species differs from all nominal species of *Blommersia* by a substantial genetic divergence and by its advertisement calls (as described below).

**Description of holotype.**—Specimen in good state of preservation, tissue sample removed from left shank. SVL 20.8 mm, for further measurements see Table 1. Body slender; head longer than wide, slightly wider than body; snout slightly truncate in dorsal and slightly rounded in lateral view, nostrils directed laterally, slightly protuberant, nearer to tip of snout than to eye; canthus rostralis distinct, slightly concave; loreal region slightly concave; tympanum distinct, rounded, its diameter 62% of eye diameter; supratympanic fold moderately distinct, slightly curved; tongue ovoid, bifid posteriorly; vomerine odontophores rounded and small, but clearly recognisable, maxillary teeth small; choanae small, rounded. Arms slender, subarticular tubercles single; fingers without webbing; relative length of fingers  $1 < 2 < 4 < 3$ ; finger disks distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches anterior eye corner when the hindlimb is adpressed along the body; lateral metatarsalia partially connected; inner and outer metatarsal tubercles distinct; webbing formula (according to Blommers-Schlösser 1979) between toes on both feet 1(1), 2i(1), 2e(0.5), 3i(1.5), 3e(1), 4i(2), 4e(2), 5(0.5); 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 positioned at the distal part of the thigh, moderately distinct, slightly elevated, measuring  $4.2 \times 1.4$  mm, of type 2 sensu Glaw *et al.* (2000), distance between femoral glands very large, 9.8 mm.

After four years in preservative, the dorsum is brown with a narrow median line running from a point between the eyes to the inguinal region and with several poorly contrasting elongated darker and largely symmetrical markings. The upper flanks are of the same colour as the dorsum, and whitish below a sharply delimited and

distinct colour border. The hindlimbs are brown with 3–4 distinct darker brown crossbands on thighs, 3–4 on shank, and 2 on tarsus. The lower arms also have two dark crossbands. Posterior to the eye the head is laterally marked by a distinct dark brown streak underneath the supratympanic fold, which includes the tympanum and ends close to the forelimb insertion. Ventrally dirty cream-yellowish, with fine indistinct mottling on throat, chest, anterior belly and hindlimbs. Femoral glands of similar colour as surrounding skin, but slightly more greyish. Colour in life was similar to that in preservative.

**Variation.**—Morphological variation of the paratypes is provided in Table 1. Dorsal colouration of the ZSM paratypes is rather variable, ranging from dark brown (ZSM 459/2006), brown with distinctly darker flanks (ZSM 457/2006), reddish with mottled brown flanks (ZSM 458/2006, female, without recognisable femoral glands) to light grey with darker spots and a thin light median line (ZSM 456/2006). Ventral colouration is similar to that of the holotype except in ZSM 459/2006 which has a distinctly brownish mottled chest. The ZMA paratypes are quite variable in colouration as well (e.g. Fig. 7), sometimes with a thin white vertebral stripe (ZMA 19504 and 19506) or dark flanks separated by a sharp dorsolateral colour border from the lighter dorsum (ZMA 19500–19501).

**Etymology.**—The species name is dedicated to Wilfried W. de Jong, Nijmegen, in recognition of his outstanding contributions to understanding the molecular phylogenetics among the main mammal lineages.

**Natural history.**—Calling specimens were observed in the rainy season at Nosy Boraha and near Toamasina in the dense vegetation over flooded areas and temporary ponds, calling from the vegetation at 10–150 cm perch height. A female specimen from near Toamasina deposited yellowish eggs, similar to other clutches that were found at the field site on leaves overhanging the water.

**Vocalisation.**—The advertisement call of *Blommersia dejongi* recorded at the type locality (Maromandia village, Nosy Boraha) on 7–8 March 2006 at an estimated air temperature of 25°C, consists of a series of moderately long, unharmonious notes, repeated singly at irregular intervals or in series at a repetition rate of approximately 8 notes/second (Fig. 8). Series are composed of 4–6 notes, resulting in call durations of 530–650 ms ( $n = 3$ ). Other numerical parameters are as follows: note duration,

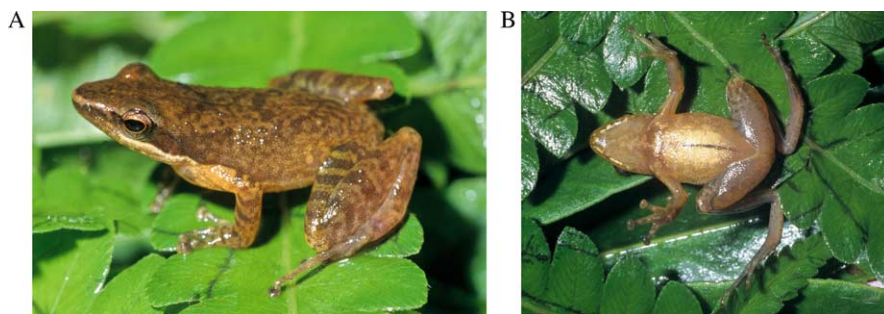


Figure 7. Male paratype of *Blommersia dejongi* sp. nov. (ZMA 19505) from Toamasina in life; (A) dorsolateral and (B) ventral view.

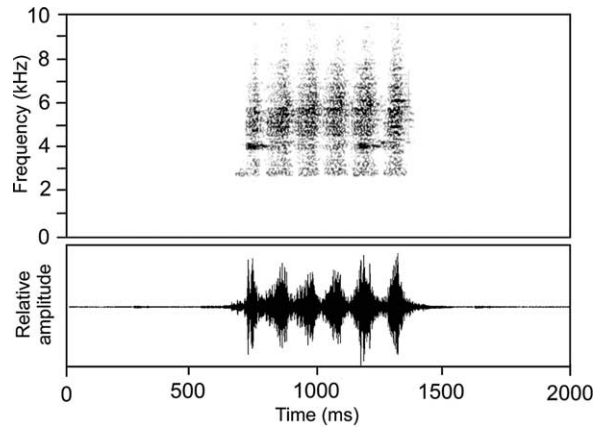


Figure 8. Spectrogram and corresponding oscillogram of the advertisement call of *Blommersia dejongi* sp. nov., recorded on 7–8 March 2006 at the type locality. High-pass filtered at 2200 Hz.

72–127 ms ( $101.6 \pm 16.7$ ;  $n = 13$ ); inter-note interval, 17–38 ms ( $26.9 \pm 7.5$ ;  $n = 11$ ); dominant frequency, 5 220–6 100 Hz ( $5 914 \pm 308$ ;  $n = 6$ ). Overall frequency is distributed in a very broad band from approximately 3 000–11 000 Hz. Within note series, notes are barely separated from each other, and depending on the recording quality sometimes even seem to be fused, with the interval barely measurable. In any case, inter-note intervals are very short in relation to the note duration. All notes appear strongly pulsed, although no separate pulses are distinguishable, or countable. Notes exhibit some amplitude modulation with highest energy present in the middle of the note.

Calls of *B. dejongi* recorded at Toamasina (Vences *et al.* 2006, CD 1, track 78) generally agree with those from Nosy Boraha (see Table 2), although the short note series recorded usually started with a very short initial note (19–30 ms;  $n = 2$ ) and notes showed an even stronger pulsatile structure.

**Comparative call data.**—The call of *B. dejongi* mainly differs from those of *B. wittei* and *B. galani* by a distinctly longer note duration and much shorter inter-note intervals (Table 2). Furthermore, the call of *B. dejongi* differs from those of other *Blommersia* species as follows (recordings available from Vences *et al.* 2006; only the most important differences summarised here): *B. kely* and *B. sarotra* emit single long notes, each composed of distinctly recognisable pulses (vs. fast series of short notes in *B. dejongi*); *B. domerguei* and *B. grandisonae* have two note types and typically one long note is followed by a series of several short notes (vs. typically only a series of short notes). The call is most similar to that of *B. blommersae* which emits a series of typically 2–3 chirps of a similar note duration as in *B. dejongi*, but repeated after slightly longer intervals ( $> 30$  ms).

**Distribution.**—*Blommersia dejongi* is known from (1) its type locality Nosy Boraha as well as from (2) a locality ca. 10 km north of Toamasina along national road no. 5, based on molecular, morphological and bioacoustic evidence. The position of these localities is shown in Fig. 6.

## DISCUSSION

### Microendemism in *Blommersia* Species

This study adds two new species to the genus *Blommersia*, both of which seem to have distinctly smaller distribution areas than most other species of the genus. *Blommersia dejongi* is known from coastal localities at a latitudinal range of about 150 km, whereas *B. galani* so far has been found at localities of a maximum distance of 110 km. Although very few surveys with appropriate integrative methodology are thus far available from higher elevations at these latitudes, our inventories from Ambodisakoa near Alaotra lake (17.31203° S, 48.66612° E, 804 m a.s.l.) and Makira forest (15°25'22.3" S, 49°07'15.1" E, 1034 m a.s.l.) only yielded *B. blommersae*, indicating that *B. dejongi* and *B. galani* are probably restricted to the relatively narrow stretch of low elevations along the coast. Furthermore, we consider it to be unlikely that these species occur much further north or south: between Toamasina and the Mangoro river, a single coastal *Blommersia* record refers to a new unconfirmed candidate species recently collected by P.-S. Gehring and F. Ratsoavina at Vatomandry (unpublished data). According to our data, south of the Mangoro the coastal swamp habitats are usually occupied by *B. blommersae*, a species that in this region descends virtually to sea level. North of Nosy Boraha, swamps at Voloina and Maroantsetra are occupied by *Blommersia* sp. 2, which will be studied more in detail in a forthcoming study; and still further north, the only coastal *Blommersia* species we could so far detect is *B. wittei*. We therefore assume that our localities indeed span the largest part of the distribution areas of *B. galani* and *B. dejongi*, which therefore together with *B. sp. 2* can be considered as microendemic species and restricted to a single centre of endemism which corresponds to CE2 of Wilmé *et al.* (2006). Since these three species appear to form a monophyletic group, they have probably diversified within this centre of endemism. Searches for *Blommersia* populations between Voloina and Nosy Boraha, and south of Toamasina, are necessary to identify their precise distribution boundaries and to ascertain their possible sympatry with *B. sp. 2*.

### Femoral Gland Structure as a Taxonomic Character in Mantellines

One of the main external characters allowing immediate identification of the two new species described herein is the structure of their femoral glands. The presence of femoral glands in mantellines is certainly related to their derived mating behaviour during which the male positions itself above the female, without amplexus, and moves with the ventral sides of his thighs over the female's head and dorsum (Blommers-Schlösser 1975). Femoral gland structure differs and is phylogenetically informative among major mantelline groups (Glaw *et al.* 2000; Vences *et al.* 2007). Furthermore, there are several examples of closely related species that differ in the size and shape of their femoral glands (Glaw & Vences 2007). In *B. galani* and *B. dejongi*, this difference is extreme, and the gland structure in *B. dejongi* (glands extending to the knee joints) is unknown in any other species in the entire family Mantellidae. It is appealing to speculate that these organs are under sexual selection, i.e. that they might produce pheromones that are used by females to select mates or to decide the number of eggs to be deposited in a specific mating. Because such

sexually selected traits often diverge rapidly between independently evolving sister lineages they can result in pre-zygotic barriers isolating these lineages (species) and thus be involved in driving species formation. To test such hypotheses, more data are necessary to understand (1) which, if any, sexual pheromones are secreted by these glands and how these differ among species of mantellines, and (2) how constant femoral gland structure is within species, both among individuals and seasonally in the same individual (e.g. during vs. outside the mating season).

## Adaptability and Conservation of Lowland Malagasy Frogs

Following the vegetation categories of Lowry *et al.* (1997) and Gautier & Goodman (2003), rainforest at low altitudes along Madagascar's east coast can be classified as (a) moist evergreen littoral forests and (b) low-elevation moist evergreen forests (0–800 m), whereas degraded and secondary formations are (c) secondary thickets, (d) secondary grasslands (savanna), and (e) cultivated areas. Our observations indicate that *Blommersia galani* and *B. dejongi*, as well as numerous other amphibian species living at moist tropical lowland sites in Madagascar, are not restricted to the two primary forest types prevailing in this area (categories a and b). In fact, it is common to observe comparatively rich amphibian communities in totally degraded habitats on Madagascar's east coast, far from any primary forest (in secondary thickets and especially in cultivated areas, habitat categories c and e). At Nosy Boraha, this applies at least to 13 species: *Anodonthyla boulengeri*, *Blommersia dejongi*, *B. galani*, *Boophis opisthodon*, *B. tephraeomystax*, *Heterixalus madagascariensis*, *H. punctatus*, *Guibemantis timidus*, *Mantella ebenaui*, *Mantidactylus* sp. aff. *betsileanus*, *Plethodontohyla notosticta*, *Ptychadena mascareniensis* and *Stumpffia tetradactyla*. Only a rather limited number of species known from the Malagasy lowlands, especially those belonging to the genera *Gephyromantis* (*G. leucomaculatus*, *G. luteus*, *G. redimitus*) and *Spinomantis* (*S. aglavei*), are clearly more restricted to primary, even if disturbed, rainforest habitat. On the contrary, in mid-altitude rainforests of eastern Madagascar, after destruction of the primary forest, only very few species are able to survive, usually *Heterixalus* spp., *Boophis tephraeomystax*, *Ptychadena mascareniensis*, and sometimes species of *Blommersia* and *Mantidactylus*, and *Boophis luteus* and *B. goudoti*.

There are various possible explanations for this main difference according to our observations. First, most areas at mid-elevations in eastern Madagascar are characterised by comparatively steep slopes on which, after slash-and-burn agriculture, all soil nutrients are quickly washed away and the soil maintains no humidity and harbours no understory vegetation with a moist leaf litter needed by most amphibians. Second, trees planted at mid-elevations are usually exotic pines and eucalypts under which no moist leaf litter appropriate for amphibians accumulates. In addition, mid-elevational savanna (habitat category d) is regularly burned by locals, again preventing the accumulation of a suitable moist layer on the soil. On the contrary, the flat coastal areas are usually characterised by a mosaic of rice fields and plantations, often with large mango trees next to villages, and with large areas that become flooded during the rainy season. Altogether this appears to provide adequate habitat for a relatively large portion of the generalists among

amphibian species occurring in lowland habitats, a hypothesis that requires more thorough testing through comparative and quantitative surveys in altered and intact habitats at various elevational levels in Madagascar.

Assessing the conservation status of all species of Madagascar's amphibians is a relevant issue for the understanding of the possible trends in the rate of their decline (Andreone *et al.* 2005, 2008). So far, no species of *Blommersia* is included in any of the threatened categories (Critically Endangered, Endangered, or Vulnerable). Although *Blommersia dejongi* and *B. galani* are known from only a small stretch of Madagascar's east coast, their adaptability to severely degraded habitats indicates that they do not face any immediate threat to the survival of their populations. Chytrid fungus, an emergent disease threatening amphibian populations worldwide with decline, is so far absent from Madagascar and the distribution areas of the two species described herein are outside of the areas bioclimatically most suited for this infection (Lötters *et al.* 2008). We therefore propose for them the status of Least Concern according to IUCN criteria (IUCN 2001).

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