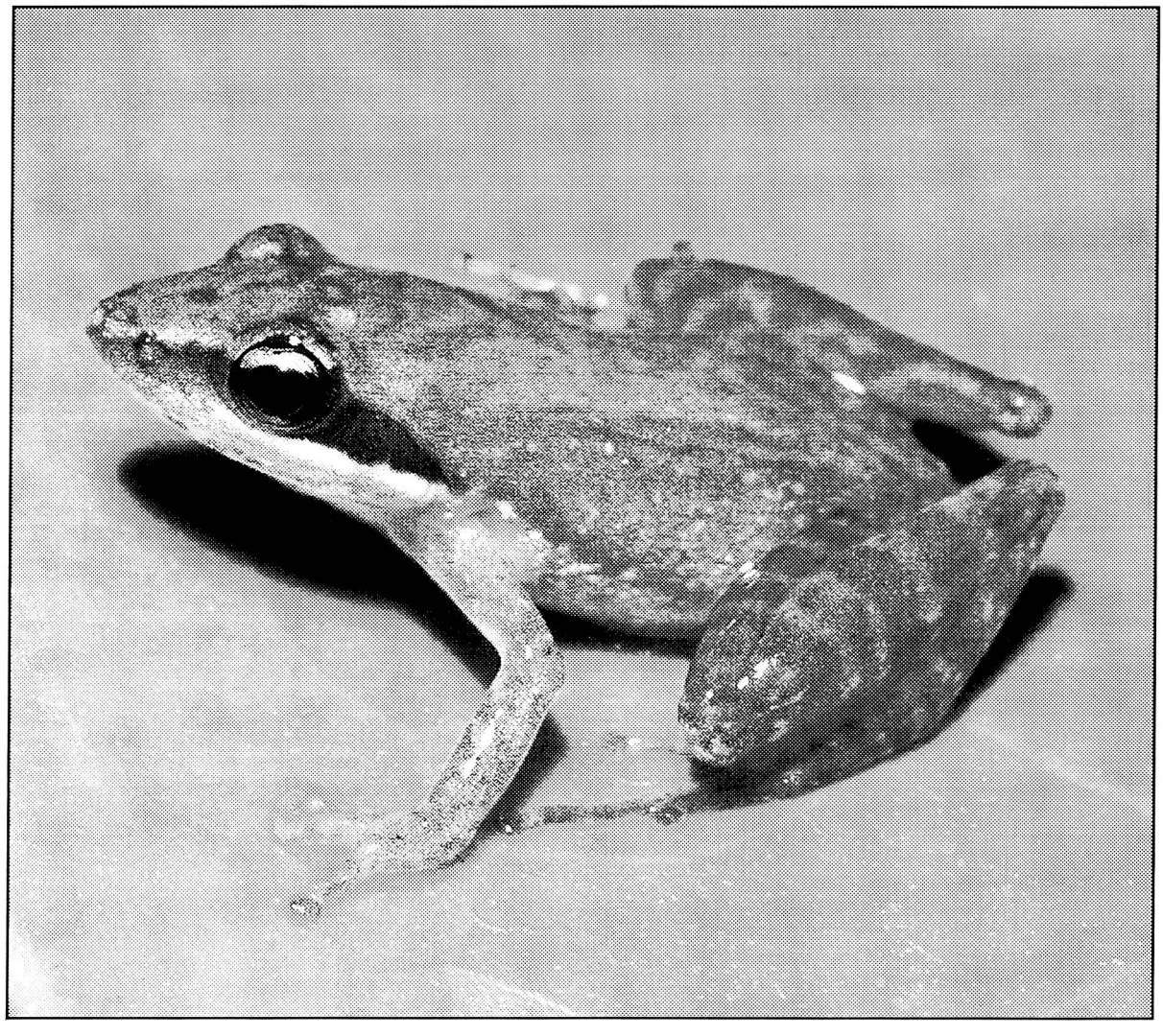
Volume 12, Number 1

January 2002

ISSN 0268-0130

# THE HERPETOLOGICAL JOURNAL



Published by the
BRITISH HERPETOLOGICAL SOCIETY

Indexed in
*Current Contents*

HERPETOLOGICAL JOURNAL, Vol. 12 , pp. 11-20 (2002)

# A NEW SIBLING SPECIES OF THE ANURAN SUBGENUS *BLOMMERSIA* FROM MADAGASCAR (AMPHIBIA: MANTELLIDAE: *MANTIDACTYLUS*) AND ITS MOLECULAR PHYLOGENETIC RELATIONSHIPS

FRANK GLAW[1] AND MIGUEL VENCES[2]

[1] *Zoologische Staatssammlung, Münchhausenstr. 21, 81247 München, Germany*

[2] *Muséum national d'Histoire naturelle, Laboratoire des Reptiles et Amphibiens, 25 rue Cuvier, 75005 Paris, France*

A new species of the *Mantidactylus domerguei* species group in the subgenus *Blommersia* is described from central eastern Madagascar. *Mantidactylus sarotra* sp. n. is morphologically similar to the syntopic *M. blommersae* but differs by smaller size, vocal sac coloration, advertisement calls and habitat of calling males. A phylogenetic analysis of 565 nucleotides of the mitochondrial 16S rRNA gene of all described species of the *M. domerguei* group revealed that *M. sarotra* and *M. blommersae* are not sister species but are genetically highly differentiated (9.73% sequence divergence). *M. sarotra* was grouped with high bootstrap support as a sister species of *M. kely*. The two species share a general advertisement call structure, vocal sac coloration and small size, but differ from each other in terms of skin texture, dorsal coloration, and pulse rate of vocalizations. The high differentiation among all species of the group (4.96% divergence between the closest relatives, *M. blommersae* and *M. domerguei*) indicate that speciation of the currently recognized taxa probably occurred several million years before the present.

*Key words*: *Mantidactylus sarotra* sp. n., *Blommersia,* Madagascar, phylogeny, mitochondrial DNA, advertisement calls

## INTRODUCTION

The speciose Malagasy genus *Mantidactylus* is a diverse assemblage of about 75 nominal species of frogs, divided into 12 subgenera. Recent molecular and morphological data indicate that it is paraphyletic relative to the well established genus *Mantella* (Richards *et al.*, 2000; pers. obs.). Subdivision of *Mantidactylus* into several genera also seems adequate considering the high morphological and biological diversity of the included species (Glaw & Vences, 1994). However, stability of such a partitioning can only be reached if the natural history and relationships of the taxa involved are satisfactorily known. One group with unresolved taxonomy in the subgenus *Blommersia* is composed of small, mainly swamp-breeding and partly arboreal frogs. It was named *Mantidactylus wittei* complex by Glaw & Vences (1994); here we define it as *Mantidactylus domerguei* species group, named after the historically first described taxon included in the group. Although rather common in eastern Madagascar, the species of the *M. domerguei* group were not discovered before the early 1970s, when Guibé (1974*a,b*, 1975) described four species: *M. blommersae, M. domerguei, M. grandisonae* and *M. wittei*. Blommers-Schlösser (1979) provided the first information on the natural history of three species (*M. blommersae, M. domerguei, M. wittei*). Glaw & Vences (1994) added information on *M. grandisonae*, described a further species (*M. kely*) and recognized two additional undescribed species (*Mantidactylus* sp. a and *M.* sp. b). During the last six years, additional information on morphological, bioacoustic and genetic differentiation of species of *Blommersia* has become available. These new data enable us to diagnose reliably one of the previously recognized new species (*M.* sp. b). In the present paper, we describe this species and assess its phylogenetic relationships to other species of the *M. domerguei* group using mitochondrial DNA sequences.

*Correspondence*: F. Glaw, Zoologische Staatssammlung,. Münchhausenstr. 21, 81247 München, Germany. *E-mail*: frank.glaw@zsm.mwn.de

## MATERIALS AND METHODS

Specimens were collected during day and night by localization of calling males. Whenever possible, we collected specimens only after identification by vocal sac inflation during call climax, to reliably link call recordings to voucher specimens. Vocalizations were recorded using portable tape recorders with external microphones and were analysed either with the MEDAV sound analysing system Spektro 3.2 or on a PC using the software CoolEdit (Syntrillium Corp.). Vouchers were fixed in 96% ethanol and subsequently stored in 70% ethanol. Museum acronyms used are MNHN (Muséum national d'Histoire naturelle, Paris), UADBA (Université d'Antananarivo, Département de Biologie Animale), ZFMK (Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn), and ZSM (Zoologische Staatssammlung, München). As no definitive catalogue numbers of UADBA specimens were available, we report here the provisional field numbers of F. Glaw and M. Vences (FG/MV) for specimens stored in this collection. The following morphological measurements were taken with dial calipers to the nearest 0.1 mm: SVL (snout-vent

length), HW (head width), HL (head length), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (nostril-nostril distance), TD (tympanum diameter), HAL (hand length), FORL (forelimb length), HIL (hindlimb length), FOL (foot length), FOTL (foot length including tarsus), FGL and FGW (length and width of femoral gland). Temporal and metric measurements are given as range, with mean ± standard deviation in parentheses. DNA was extracted from muscle tissue samples preserved in ethanol. We sequenced a fragment of the mitochondrial 16S rRNA gene comprising up to 565 nucleotides (nt). Detailed information on DNA extraction, primers, PCR and sequencing are given in Vences *et al.* (2000*b*). Alignment required the inclusion of gaps in the sequences of one or more taxa at a total of 16 nucleotide sites, mainly to account for divergent sequences of outgroups in the hypervariable loop regions. Prior to phylogenetic reconstruction, we looked at which substitution model fits our sequence data the best. We applied a hierarchical likelihood ratio test for testing the goodness-of-fit of nested substitution models using the program MODELTEST (Posada & Crandall, 1998). Phylogenetic analyses were carried out using PAUP* (Swofford, 2001). We performed heuristic searches using the Maximum Likelihood (ML) method under the substitution model proposed by MODELTEST, and with random addition sequences with 10 replications and tree bisection reconnection (TBR) branch-swapping. Additionally, we calculated Maximum Parsimony (MP) cladograms with gaps treated as a fifth character, and Neighbor-joining (NJ) trees with LogDet distances which are robust against possible variation of sequence evolution among lineages (Lockhart *et al.*, 1994).

The analyses were repeated after exclusion of two hypervariable regions (spanning over a total of 62 nucleotides) and of the only two additional sites with gaps in one or more taxa in the alignment. Two-thousand bootstrap replicates were run in all analyses. The robustness of nodes was tested by Kishino-Hasegawa tests (Kishino & Hasegawa, 1989) as implemented in PAUP* (RELL bootstrap, 1000 replicates, one-tailed test).

Sequences were submitted to Genbank (see appendix for accession numbers). The following specimens were used for genetic analysis: *Boophis tephraeomystax*, ZFMK 66685 (Cap Est), Genbank accession AF215333; *Mantella laevigata*, ZFMK 65637 (no locality data), AF215280; *Mantidactylus blommersae*, UADBA-FG/MV 2000.65 (Andasibe), AF317688; *Mantidactylus depressiceps*, ZFMK 60131 (Andasibe), AF215326; *Mantidactylus domerguei*, ZSM 370/2000 (Manjakatompo), AF317689; *Mantidactylus grandisonae*, ZFMK 66669 (Ambatobe), AF215315; *Mantidactylus kely*, ZSM 363/2000 (Ambatolampy), AF317690; *Mantidactylus liber*, ZSM 491/2000 (Montagne d'Ambre), AF317686; *Mantidactylus sarotra*, ZSM 354/2000 (Mandraka), AF317687; *Mantidactylus wittei*, UADBA-FG/MV 2000.123 (Ambanja), AF317691.

## RESULTS

### A New Species of *Mantidactylus*

Field data gathered during 1995, 1996 and 2000 supported the observations of Glaw & Vences (1994) regarding the syntopic occurrence of two forms which morphologically corresponded to the description of *Mantidactylus blommersae* (Guibé, 1975). Morphological examination (Table 1) revealed no differences between them except size. Re-examination of the type series of *M. blommersae* (Table 1) corroborated that holotype and paratypes agree in size and morphology with the larger form which had already been defined as *M. blommersae* by Glaw & Vences (1994). The female holotype and paratypes were not conspicuously larger than the male paratypes, as usual in many *Mantidactylus* (Blommers-Schlösser, 1979). The smaller form corresponds to an undescribed form, designated *Mantidactylus* sp. b in Glaw & Vences (1994).

Males of *M. blommersae* generally called from the underside of leaves 10-20 cm above swamps, while specimens of the undescribed species (Figs. 1,2) called from positions near or on the ground, very well hidden in deep vegetation, and generally not directly from structures above the water surface. Vocalizations of the two were consistently different: *M. blommersae* emit-

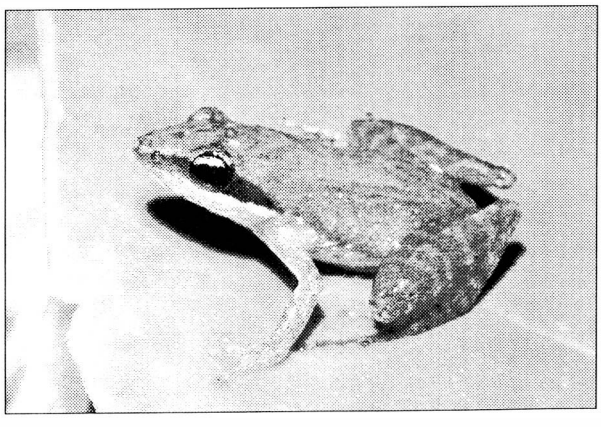


FIG. 1. Dorsolateral view of *Mantidactylus sarotra* (male holotype) in life.

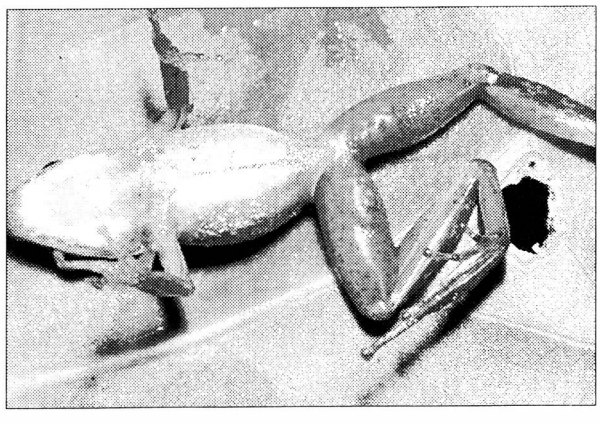


FIG. 2. Ventral view of *Mantidactylus sarotra* (male holotype) in life.

TABLE 1. Morphometric measurments (all in mm) of specimens of *Mantidactylus sarotra, M. blommersae* and *M. kely*. For abbreviations of measured variables, see Materials and Methods; further abbreviations used: M (male), F (female), HT (holotype), PT (paratype), RHL (relative hindlimb length: point reached by tibiotarsal articulation when the hindlimb is adpressed along the body: 0, anterior eye margin; 1, between eye and nostril; 2, nostril; 3, snout tip).

| Catalogue number | Status | Sex | Locality | SVL | HW | HL | TD | ED | END | NSD | NSD | FORL | HAL | HIL | FOTL | FOL | FGL | FGW | RHL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *M. sarotra* | | | | | | | | | | | | | | | | | | | |
| ZFMK 62887 | PT | M | Mandraka | 15.2 | 4.7 | 6.0 | 1.1 | 2.0 | 1.3 | 1.2 | 1.7 | 10.0 | 4.4 | 27.7 | 12.6 | 7.8 | 3.0 | 1.3 | 2 |
| ZSM 351/2000 | HT | M | Mandraka | 16.8 | 5.0 | 6.6 | 1.1 | 2.0 | 1.6 | 1.5 | 1.9 | 12.5 | 4.9 | 30.5 | 13.9 | 8.7 | 3.0 | 1.4 | 3 |
| ZSM 354/2000 | PT | M | Mandraka | 15.7 | 4.8 | 6.0 | 1.2 | 1.9 | 1.4 | 1.1 | 1.9 | 10.6 | 4.6 | 28.6 | 13.4 | 8.7 | 2.4 | 1.3 | 2 |
| *M. kely* | | | | | | | | | | | | | | | | | | | |
| ZFMK 57444 | HT | M | Manjakatompo | 15.3 | 5.0 | 6.0 | 0.8 | 2.3 | 1.5 | 1.2 | 1.9 | 10.3 | 4.0 | 24.7 | 12.4 | 8.1 | 2.8 | 1.3 | 1 |
| ZSM 363/2000 | - | M | Ambatolampy | 14.2 | 4.0 | 5.6 | 0.9 | 1.7 | 1.1 | 1.0 | 1.6 | 8.8 | 3.5 | 22.9 | 11.0 | 7.2 | 2.9 | 1.0 | 0 |
| ZSM 364/2000 | - | M | Manjakatompo | 15.9 | 4.7 | 6.0 | 0.9 | 2.3 | 1.4 | 1.2 | 1.7 | 10.4 | 4.0 | 27.0 | 12.8 | 8.3 | 2.8 | 1.4 | 1 |
| *M. blommersae* | | | | | | | | | | | | | | | | | | | |
| MNHN 1975.05 | HT | F | 25 km S Moramanga | 20.1 | 6.0 | 7.6 | 1.3 | 2.3 | 1.8 | 1.4 | 2.4 | 12.5 | 5.1 | 34.0 | 15.7 | 9.9 | - | - | 1 |
| MNHN 1975.06 | PT | F | Ranomafana | 19.6 | 5.6 | 7.4 | 1.4 | 2.2 | 1.9 | 1.3 | 2.3 | 12.0 | 4.9 | 30.3 | 14.4 | 9.5 | - | - | 0 |
| MNHN 1975.07 | PT | M | Andasibe | 21.3 | 6.7 | 8.2 | 1.2 | 2.4 | 1.9 | 1.6 | 2.4 | 14.1 | 6.4 | 36.9 | 16.7 | 11.2 | 3.2 | 1.5 | 1 |
| MNHN 1975.08 | PT | M | Andasibe | 20.2 | 6.1 | 7.3 | 1.2 | 2.5 | 1.8 | 1.5 | 2.3 | 14.0 | 6.2 | 34.9 | 17.2 | 11.2 | 3.0 | 1.3 | 1 |
| MNHN 1975.09 | PT | M | Andasibe | 18.4 | 6.0 | 7.0 | 1.6 | 2.2 | 1.5 | 1.4 | 2.2 | 11.8 | 5.4 | 31.2 | 14.9 | 9.8 | 3.9 | 1.4 | 2 |
| MNHN 1975.10 | PT | M | Andasibe | 18.8 | 6.0 | 7.6 | 1.3 | 2.2 | 1.8 | 1.3 | 2.3 | 14.3 | 6.5 | 34.1 | 15.8 | 10.6 | 3.3 | 1.2 | 2 |
| MNHN 1975.11 | PT | F | Andasibe | 20.4 | 6.3 | 8.1 | 1.4 | 2.2 | 1.6 | 1.5 | 2.1 | 11.5 | 5.0 | 32.4 | 15.1 | 9.7 | - | - | 0 |
| MNHN 1975.12 | PT | F | Andasibe | 19.3 | 5.6 | 7.6 | 1.4 | 2.1 | 1.9 | 1.5 | 2.3 | 11.7 | 4.8 | 28.7 | 13.0 | 8.3 | - | - | 0 |
| MNHN 1975.13 | PT | M | Andasibe | 19.0 | 5.6 | 6.9 | 1.3 | 2.2 | 1.5 | 1.3 | 2.0 | 12.3 | 5.4 | 31.7 | 14.8 | 9.4 | 2.8 | 1.4 | 2 |
| ZFMK 59819 | - | F | Andasibe | 19.3 | 5.3 | 6.9 | 1.1 | 2.1 | 1.6 | 1.5 | 2.1 | 12.2 | 4.8 | 31.1 | 14.4 | 9.1 | - | - | 0 |
| ZFMK 59877 | - | M | Andasibe | 19.2 | 5.8 | 7.3 | 1.2 | 2.4 | 1.7 | 1.5 | 2.2 | 13.0 | 5.8 | 31.8 | 15.2 | 9.6 | 3.9 | 1.7 | 1 |
| ZFMK 59878 | - | M | Andasibe | 19.4 | 6.0 | 7.1 | 1.3 | 2.3 | 1.6 | 1.4 | 2.4 | 13.2 | 5.6 | 33.6 | 15.2 | 9.8 | 2.6 | 1.4 | 2 |
| ZFMK 62226 | - | M | Andasibe | 21.0 | 6.2 | 7.5 | 1.3 | 2.2 | 1.7 | 1.5 | 2.1 | 13.3 | 5.9 | 32.6 | 15.4 | 10.2 | 4.2 | 1.4 | 0 |
| ZFMK 62227 | - | M | Andasibe | 20.0 | 5.8 | 7.2 | 1.4 | 2.3 | 1.6 | 1.5 | 2.0 | 13.0 | 5.5 | 33.3 | 15.8 | 10.2 | 3.8 | 1.5 | 0 |
| ZFMK 62228 | - | M | Andasibe | 19.5 | 6.1 | 7.5 | 1.5 | 2.3 | 1.7 | 1.5 | 2.0 | 12.3 | 5.7 | 33.2 | 15.2 | 10.0 | 3.5 | 1.5 | 1 |
| ZFMK 62229 | - | M | Andasibe | 20.0 | 6.0 | 7.5 | 1.2 | 2.0 | 1.6 | 1.4 | 2.2 | 12.5 | 5.2 | 31.8 | 14.6 | 9.1 | 3.5 | 1.6 | 0 |
| ZFMK 62230 | - | M | Andasibe | 18.6 | 5.7 | 7.8 | 1.4 | 2.4 | 1.6 | 1.5 | 2.2 | 12.2 | 5.4 | 31.7 | 14.0 | 9.1 | 3.2 | 1.2 | 2 |
| ZFMK 62231 | - | M | Andasibe | 19.4 | 6.0 | 7.2 | 1.4 | 2.2 | 1.6 | 1.4 | 2.1 | 12.7 | 5.2 | 32.5 | 15.1 | 9.7 | 3.4 | 1.3 | 1 |
| ZFMK 62232 | - | M | Andasibe | 19.9 | 6.2 | 7.5 | 1.2 | 2.2 | 1.6 | 1.5 | 2.3 | 12.9 | 5.7 | 33.6 | 15.5 | 10.4 | 3.5 | 1.7 | 1 |
| ZFMK 62233 | - | M | Andasibe | 18.0 | 5.5 | 6.7 | 1.2 | 2.0 | 1.6 | 1.4 | 2.0 | 12.2 | 5.5 | 30.4 | 13.8 | 9.1 | 3.7 | 1.6 | 1 |

ted series of two to three similar short chirp notes (Table 2). In contrast, the undescribed species had three different note types: two longer pulsed notes and a short click note, combined in different ways (Table 3). Capture of specimens of the undescribed species was extremely difficult; only at one locality (Mandraka) did we succeed in capturing four male specimens which were all observed during call emission. At a nearby locality, Andasibe, we captured a further eight male specimens of *M. blommersae* (ZFMK 62226-62233) which were all observed emitting their typical calls.

DNA sequences were obtained from one specimen of each species in the *M. domerguei* group. The resulting phylograms (Fig. 3) did not group the two *M. blommersae*-like forms as sister species. Instead, *M. blommersae* was the sister species of *M. domerguei*, and the undescribed species was the sister species of *M. kely*. Bootstrap support for these groupings was high (89-98% in all cases). Alternative trees with *M. blommersae* and the undescribed species as sister groups needed 24 or 25 additional steps and were significantly worse under both Maximum Parsimony and Maximum Likelihood models (Kishino-Hasegawa tests; $P<0.05$). Analyses after exclusion of all hypervariable (loop) regions resulted in slightly lower support for the relevant groupings (bootstrap values 76-92%), but the alternative topologies could still be significantly excluded by Kishino-Hasegawa tests ($P<0.05$).

The undescribed species differed from its closest relative *M. kely* by 35 nucleotides (not considering indels) in the considered fragment (6.20%), while it differed from *M. blommersae* by 55 nt (9.73%) (Table 4); *M. blommersae* differed by 28 nt (4.96%) from its sister species *M. domerguei*. The high genetic differentiation and phylogenetic position of the undescribed species leave no doubt as to its distinctness; we therefore describe it formally in the following.

*MANTIDACTYLUS SAROTRA* SP. N. (FIGS. 1,2)

*Diagnosis.* A species assigned to the genus *Mantidactylus* based on the absence of nuptial pads and presence of femoral glands in males. Assigned to the *M. domerguei* group in the subgenus *Blommersia* based on small size, low relative hand length (ratio HAL/SVL <30%), a white horseshoe shaped marking on the throat, single subgular vocal sac, femoral gland morphology (single patch of similarly sized granules), and relatively elongated head (ratio HW/HL 76-80%). Within the *M. domerguei* group, *M. sarotra* is distinguished from *M. grandisonae, M. domerguei* and *M. blommersae* by a distinct white horseshoe shaped marking on the throat and by general structure of advertisement calls. It is further distinguished from *M. wittei*

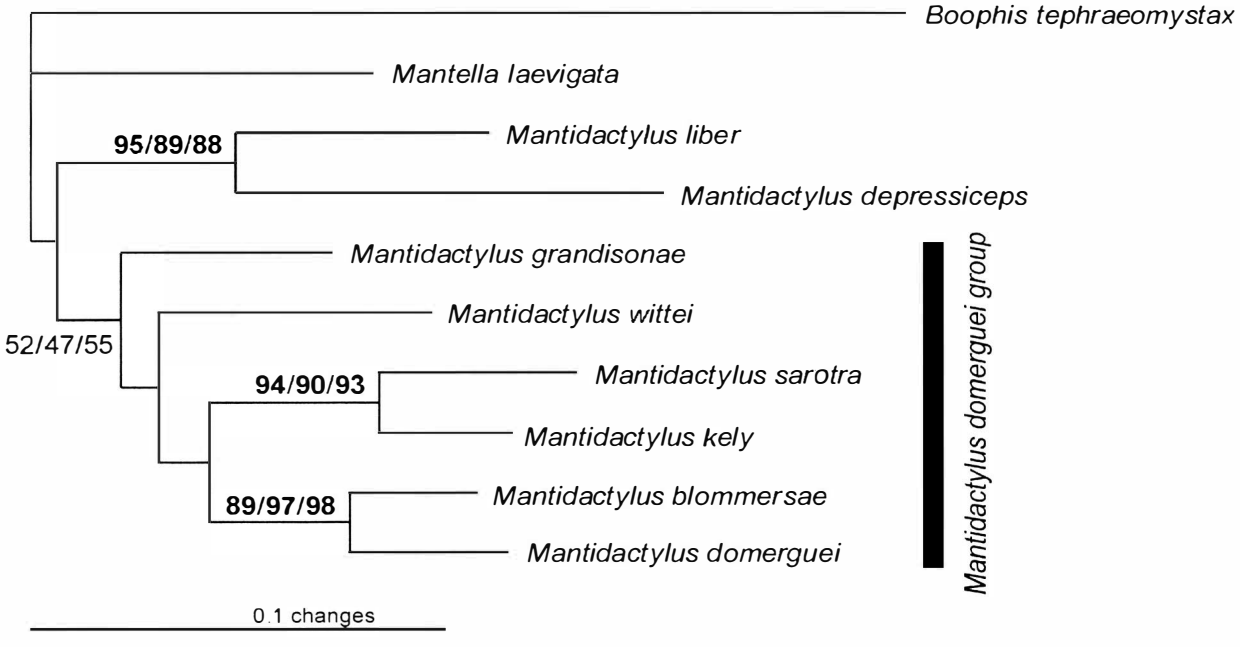



FIG 3. Maximum Likelihood phylogram based on 565 nucleotides of a fragment of the mitochondrial 16S rRNA gene in species of the *Mantidactylus domerguei* group, obtained using settings estimated by the program MODELTEST: Tamura-Nei substitution model with empirical base frequencies (A: 0.3154; C: 0.2237; G: 0.1833; T: 0.2776) and substitution rates (A-G: 2.7672; C-T: 8.7501; all other rates: 1), no invariable sites and a gamma distribution shape parameter of 0.1648. The topology agrees with that of the most parsimonious tree found by Maximum Parsimony analysis (396 constant and 97 parsimony-informative characters; 345 steps; consistency index 0.67, retention index 0.45) except for the arrangement of clades within the *M. domerguei* group (*M. wittei* placed as sister group of the *M. blommersae/M. domerguei* clade). Numbers at nodes are bootstrap support (2000 replications) in percent for Maximum Likelihood (left), Maximum Parsimony (middle) and Neighbour-joining (using LogDet distances; right) analyses, respectively. Bootstrap values below 50% in all three analyses are not shown. *Boophis tephraeomystax* was used as outgroup.

TABLE 2. Temporal and spectral call parameters in various populations of *Mantidactylus blommersae*.

| | Ranomafana | Ankeniheny | An'Ala |
|---|---|---|---|
| Recording temperature | 22°C | 20.5°C | 22°C |
| Recording date | 29 February 1996 | 19 February 1994 | 11 February 1995 |
| Note duration [ms] | 29-65 (47±11,*n*=14) | 45-129 (69±31,*n*=6) | 113-187 (138±23,*n*=10) |
| Duration of interval between notes [ms] | 26-52 (39±11,*n*=4) | 42-49 (46±4,*n*=3) | 13-33 (21±7,*n*=7) |
| Frequency [Hz] | 3550-8850 | 4000-7100 | 4100-8300 |
| Dominant freq. [Hz] | 5900-6700 | 5800 | 5900 |

TABLE 3.Temporal and spectral call parameters in various populations of *Mantidactylus sarotra* and *M. kely*. Missing data refer to note types which may occur in the corresponding populations, but which were not recorded.

| | *M. sarotra* | *M. sarotra* | *M. sarotra* | *M. kely* | *M. kely* |
|---|---|---|---|---|---|
| *Recording information* | | | | | |
| Locality | Mandraka | Andasibe | Ranomafana | Manjakatompo | Ambatolampy |
| Temperature | 18.4°C | 25.5°C | not recorded | 18°C | 23.2°C |
| Date | 8 Feb. 2000 | 30 Jan. 1996 | 28 Feb. 1996 | 8 Jan. 1994 | 11 Feb. 2000 |
| *Notes of type 1* | | | | | |
| Note duration [ms] | 187-301 (244±80,*n*=2) | 244-256 (250±6,*n*=3) | 266-370 (321±34,*n*=10) | 453-637 (564±60,*n*=10) | 247-510 (399±91,*n*=10) |
| Number of pulses per note | 35-55 (45±14,*n*=2) | 44-46 (45±1,*n*=2) | 37-50 (44±4,*n*=10) | 23-36 (28±4,*n*=10) | 19-35 (28±6,*n*=10) |
| Pulse repetition rate [1/s] | 183-187 (185±3,*n*=2) | 180 (*n* = 2) | 130-145 (138±5,*n*=10) | 39-61 (50±7,*n*=10) | 55-84 (71±8,*n*=10) |
| *Notes of type 2* | | | | | |
| Note duration [ms] | 96-138 (124±19,*n*=4) | 118-172 (143±17,*n*=13) | 87-202 (116±48,*n*=5) | - - | - - |
| Number of pulses per note | 21-27 (24±3,*n*=4) | 19-25 (23±2,*n*=6) | 15-31 (19±7,*n*=5) | - | - |
| Pulse repetition rate [1/s] | 181-218 (198±19,*n*=4) | 136-182 (165±16,*n*=6) | 153-185 (170±12,*n*=5) | - | - |
| *Notes of type 3* | | | | | |
| Note duration [ms] | 11-14 (12±1,*n*=6) | 5-13 (9±2,*n*=16) | 4-7 (6±1,*n*=5) | - - | 15-42 (24±8,*n*=10) |
| *Interval duration* | | | | | |
| between notes of types 1 and 2 [ms] | 1126-1623 (1380±177,*n*=5) | 751-1198 (912±158,*n*=13) | 741-970 (890±70,*n*=11) | 1565-2639 (1955±423,*n*=9) | 1059-1485 (1266±230,*n*=4) |
| between notes of type 1 or 2 and notes of type 3 [ms] | 29-43 (37±6,*n*=6) | 22-44 (34±6,*n*=16) | 41-50 (47±4,*n*=5) | - - | 3-33 (18±11,*n*=10) |
| *Frequency* | | | | | |
| Frequency (of notes of type 1 & 2) [Hz] | 2500-5750 | 2750-5350 | 3550-5850 | 3100-4650 | 3500-7000 |
| Dominant frequency (of notes of type 1 &2) [Hz] | 3750-5500 | 4000-5050 | 4350-4900 | 4100-4300 | 4650-4750 |

TABLE 4. Genetic differentiation between species in the *Mantidactylus domerguei* group. The values below the diagonal are numbers of pairwise substitutions in a fragment of the 16S rRNA gene (565 nucleotides); the values above the diagonal are total pairwise sequence divergences in percent. Indels were not considered.

| | *M. sarotra* | *M. blommersae* | *M. domerguei* | *M. grandisonae* | *M. kely* | *M. wittei* |
|---|---|---|---|---|---|---|
| *M. sarotra* | - | 9.73 | 9.22 | 7.61 | 6.20 | 10.09 |
| *M. blommersae* | 55 | - | 4.96 | 8.14 | 7.79 | 8.50 |
| *M. domerguei* | 52 | 28 | - | 9.20 | 8.67 | 8.14 |
| *M. grandisonae* | 43 | 46 | 52 | - | 8.85 | 7.43 |
| *M. kely* | 35 | 44 | 49 | 50 | - | 9.03 |
| *M. wittei* | 57 | 48 | 46 | 42 | 51 | - |

by absence of vomerine teeth (vs. presence) and smaller size (SVL 15-17 mm vs. 21-26 mm); from *M. grandisonae* by smaller size (SVL 15-17 mm vs. 18-23 mm) and by a uniformly brownish flank with only an interrupted line of indistinct white spots (vs. a sharp contrast between a continuous upper blackish and lower white line along the flanks); from *M. domerguei* by a uniform dorsum with only a poorly contrasted Y-shaped marking (vs. a contrasted pattern of three longitudinal dorsal bands); and from *M. blommersae* by smaller size (SVL 15-17 mm vs. 18-21 mm). By molecular analysis the closest known relative of *M. sarotra* is *M. kely*, which furthermore is the only species of the group sharing a white horseshoe shaped marking on the throat, and an advertisement call composed of a long and distinctly pulsed note which is followed by a short click note. However, the pulsed note of *M. kely* has a lower pulse repetition rate at similar temperatures (Table 3), and *M. sarotra* differs from *M. kely* by a different dorsal coloration (largely uniform light brown with only a poorly contrasted Y-shaped marking vs. dark brown with distinct blackish markings and a distinct yellowish vertebral stripe) and skin texture (smooth vs. granular).

*Etymology*. Derived from *sarotra* (Malagasy: difficult), making allusion to the difficulties involved both in capturing and correctly diagnosing the new species. The name is used as an invariable noun standing in apposition to the generic name.

*Holotype*. ZSM 351/2000, adult male, collected by F. Glaw and M. Vences on 8 February 2000 at Mandraka (18° 54' 44" S, 47° 54' 52" E, 1425 m altitude), central eastern Madagascar.

*Paratypes*. ZFMK 62887, adult male, collected by F. Glaw and M. Vences on 9 February 1994 at the type locality; ZSM 354/2000 and UADBA-FG/MV 2000.22, two adult males, collected by F. Glaw and M. Vences on 8 February 2000 at the type locality.

*Description of the holotype*. SVL 16.8 mm. For measurements, see Table 1. Body slender; head distinctly longer than wide, not wider than body; snout slightly pointed in dorsal and lateral views, nostrils directed laterally, not protuberant, nearer to tip of snout than to eye; canthus rostralis indistinct, straight; loreal region concave; tympanum distinct, rounded, 55% of eye diameter; supratympanic fold rather indistinct, slightly curved; tongue ovoid, slightly bifid posteriorly; vomerine teeth absent, maxillary teeth present; choanae rounded. Arms slender, subarticular tubercles single; metacarpal tubercles not visible; fingers without webbing; relative length of fingers 1<2<4<3, finger 2 distinctly shorter than finger 4; finger disks distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches snout tip; lateral metatarsalia connected; inner and outer metatarsal tubercles of similar size, small but distinct; only rudimentary webbing between toes; two phalanges of fifth toe (toe disk not counted)of web.

Skin on the upper surface smooth, without folds or ridges. No distinct enlarged tubercles in the cloacal region; ventral skin uniformly smooth. Femoral glands distinct, of type 2 sensu Glaw *et al.* (2000), consisting of 10 granules (diameter 0.4-0.7 mm) in internal view (after reflection of skin).

After about seven months in preservative, the back is light greyish brown with slightly darker, poorly contrasted though well-delimited pattern: a Y-shaped marking on the central dorsum and smaller spots and markings on the head and posterior dorsum. Also the flanks are slightly darker, although there is no distinct colour border between dark flanks and light back as in most *Mantidactylus* species of the subgenus *Chonomantis* or in several representatives of the genus *Mantella*. The hindlimbs are greyish brown with light crossbands: six on femur, five to six on tibia, up to eight on tarsus and foot including longest toe. 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 sickle-like close to the forelimb insertion. Anterior to the eye, a narrower dark streak is positioned underneath the canthus rostralis. Ventrally uniformly cream-white except a very fine dark mottling along the lower lip and in the chest region, and few very fine dark spots on the hindlimbs. Colour in life was similar, except a distinct frenal stripe that ran below the dark lateral head marking, from the forelimb insertion along the upper lip to a point slightly anterior to the eye (not clearly recognizable in preservative). The general colour was light brown instead of greyish brown. The iris was dark in its lower two thirds, light yellowish brown in its upper third. Ventrally, the belly was rather translucent with a

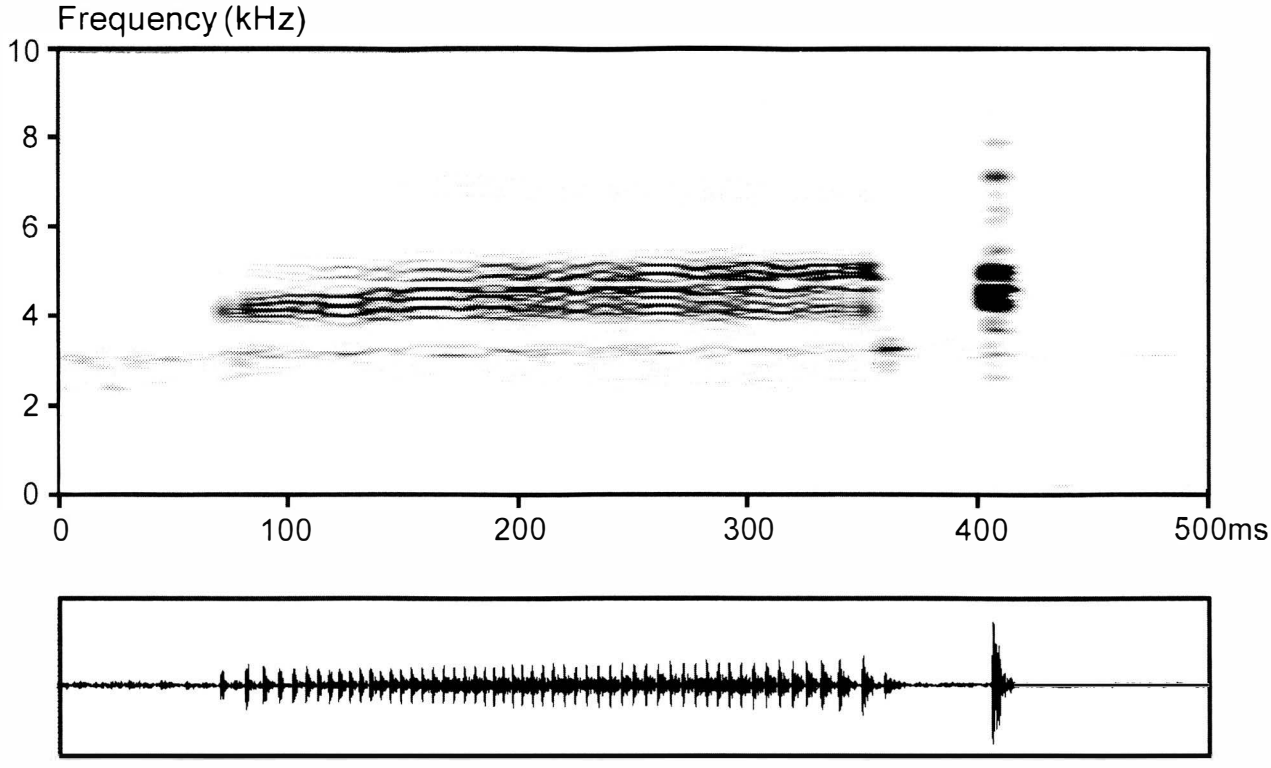



FIG. 4. Sonagram and oscillogram of a note of type 1 (left) and type 3 (right) of *Mantidactylus sarotra* (holotype) from Mandraka, recorded at 18.4°C. Notes of type 2 are similar to those of type 1 as shown in the sonagram, but have a lower number of pulses and therefore a shorter duration (see Table 3).

white central area. The throat was silvery white except for a pigmentless translucent central area.

*Variation.* The holotype and the three paratypes are very uniform in morphology and coloration (see Table 1 for measurements).

*Natural history.* Calls of *M. sarotra* were mainly heard during the day, at dusk, and early at night. Before dusk it was impossible to observe calling males, which obviously emitted their vocalizations hidden in the leaf litter or between the thin roots of the vegetation. At night, specimens were observed at Mandraka calling from positions a few centimetres above the ground close to a very small, slow-flowing ditch (10 cm wide, less than 3 cm deep). The inflated vocal sac was conspicuously white. Each note of type 1 or 2 was one expiration. Clutches possibly belonging to this species had been found attached to fallen leaves on the ground in a desiccated puddle (Glaw & Vences, 1994).

*Vocalizations.* Advertisement calls were recorded at Mandraka on 8 February 2000, at Andasibe on 30 January 1996, and at Ranomafana on 28 February 1996. Three note types could be distinguished. Notes of type 1 are unharmonious and distinctly pulsed (Fig. 4). They are often emitted at the beginning of a call. Notes of type 2 are similar to those of type 1, but of shorter duration. They are often emitted in series of 4-6 notes. Notes of type 3 are short clicks and often follow immediately after notes of type 1 or 2. A typical call is arranged as series of notes of the three types as follows: 1-3—2-3—2-3—2-3—2-3. Combinations as 1—1—1—1—1 or 1—1—1—1-3—1 can also occur. Temporal and spectral characteristics are given in Table 3.

*Distribution.* Reliably identified specimens were collected only at the type locality, Mandraka. Call records exist from Andasibe, the swamp Antorotorofotsy north of Andasibe, near Moramanga, and Ranomafana. Glaw & Vences (1994) mention a further locality, Tolagnaro in SE-Madagasçar for *Mantidactylus* sp. b. The corresponding vouchers (ZFMK 53698-53699 from Manantenina, close to Tolagnaro) are larger than the type specimens (17-20 mm SVL) and show a different coloration. As no reliable call recordings are available from this locality, we do not consider the vouchers as conspecific with *M. sarotra.* Their status pends further study.

### NEW DATA ON *MANTIDACTYLUS BLOMMERSAE*

Occurrence of *Mantidactylus blommersae* could be confirmed at the localities Ranomafana, Andasibe, Ankeniheny and Mandraka (Blommers-Schlösser & Blanc, 1991; Glaw & Vences, 1994). The species was also found at An Ala, a further mid-altitude locality in central eastern Madagascar. Calls from these localities were similar to each other. They were always composed of only a single chirp-like note type without regularly

pulsed structure, notes being arranged in series of two or three notes. Specific assignation of the populations from the Chaînes Anosyennes (Blommers-Schlösser & Blanc, 1991) is currently uncertain. Locality of the holotype is 25 km south of Moramanga (see also Blommers-Schlösser & Blanc, 1991). The paratypes have all been stated to originate from Andasibe (Guibé, 1975; Blommers-Schlösser & Blanc, 1991), but according to the MNHN catalogue one paratype (MNHN 1975.06) appears to have been collected at Ranomafana.

NEW DATA ON *MANTIDACTYLUS KELY*

*Mantidactylus kely* has so far only been reported from the type locality in the high altitude forest of Manjakatompo (19°21'30"S, 47°18'50"E; altitude ca. 1700 m). In February and March 2000, we heard the typical calls of this species also in an unforested swamp area close to Ambatolampy, about 15 km from Manjakatompo (19°21'54"S, 47°26'01"E, altitude 1595 m). Males were calling during the day from the dense vegetation along stagnant-water ditches. Recording temperature at this site was higher than at Manjakatompo, but pulse repetition rate in notes was still distinctly lower than in *M. sarotra* (Table 3). An explanation of the higher pulse rate of *M. sarotra* by temperature effects could thus be excluded. Tadpoles collected in the same water bodies could be assigned to *M. kely* by DNA sequences; they were of the generalized type typical for the *M. domerguei* group (Blommers-Schlösser, 1979) and will be described in detail elsewhere. We also noted a possible difference in the diel activity rhythms of syntopic *M. kely* and *M. domerguei* at Manjakatompo. On the six days of observation in March 2000, *M. kely* calls were mainly heard during the day. In contrast, *M. domerguei* calls were almost exclusively nocturnal, although they were often heard during the day at other localities. Only at dusk did we hear simultaneous calling of both species. The new analysis of the recordings from Manjakatompo revealed that *M. kely* also produces click notes (type 3). These were always heard at the end of the long pulsed notes (type 1). In some cases, the click was merely a slightly different final pulse; in other cases, it was a double pulse or a distinct, long click-note; this variability accounts for the high standard deviations of the corresponding temporal measurements (Table 3).

*Mantidactylus kely* was described as having separated lateral metatarsalia. This state, which does not refer to the metatarsal bones but to the investing tissue, was the main character used to distinguish the artificial genera *Gephyromantis* and *Mantidactylus* sensu Guibé (1978). Blommers-Schlösser (1979) doubted the phylogenetic value of this character and joined all involved species in one genus, *Mantidactylus*. The subgenus *Blommersia* contains species with apparently separated (*M. grandisonae, M. wittei*) and connected (*M. blommersae, M. domerguei*) metatarsalia. In the new material of *M. kely* available to us, the state of the metatarsalia is difficult to assess, and the decision (connected or separated) depends on the method of examination. Pulling the fifth toe laterally and using strong light, the metatarsalia appear separated, but without this drastic method, they appear connected. The same is true in *M. sarotra*. This indicates that the states of this character are not unequivocal in the small species of the subgenus *Blommersia*, and corroborates their doubtful phylogenetic value in the genus *Mantidactylus*.

## DISCUSSION

This paper presents the first data on the degree of genetic differentiation among sibling species of Malagasy anurans. All species of the *M. domerguei* group are remarkably similar in morphology, but well distinguished by advertisement calls. Their similar general appearance strongly suggests their monophyletic origins; nevertheless, monophyly was only weakly corroborated by the molecular data.

The phylogenetic relationships of the included species as presented here were recovered from mitochondrial sequences of single specimens, as is usual in similar analyses (e.g Richards *et al.*, 2000). The main assumption in such studies is that the mitochondrial gene tree represents the phylogenetic species tree, which means that such factors as haplotype polymorphism and recent introgression by hybridization play no relevant roles. Although a few examples of high intrapopulational mitochondrial haplotype polymorphism appear to exist in other organisms, in most cases such phenomena are uncommon (Avise, 2000). In the extensive amphibian data set available through Genbank and our own research (except for hybridogenetic species), no examples of intrapopulational polymorphism affecting more than only a few mutations in the rather conservative 16S gene are known to us. In *Blommersia,* we obtained additional sequences of three *M. wittei* populations and of additional specimens of both *M. kely* and *M. domerguei* (to be included in forthcoming publications), which corresponded to the sequences included in the present study. We therefore believe that the degree of haplotype polymorphism in this group is low, and that the mitochondrial gene tree actually corresponds to the species tree. Considering the high degree of differentiation among all taxa, any possible introgression must have occurred soon after the main cladogenetic events. If the recovered phylogeny represents correctly the relationships of species of *Blommersia,* some conclusions regarding speciation and its timing in this group are possible.

First, the genetic differentiation revealed by the DNA sequences was higher than expected in a group of such morphologically similar species. Pleistocene glaciations led to modifications in the distribution of vegetation types in Madagascar (Burney, 1996). For

example, the currently isolated montane floras were in broad contact along the central mountain chain. Two species of the *M. domerguei* group (*M. domerguei* and *M. kely*) occur mainly at higher altitudes (up to 1700-2000 m). Hence, it could be hypothesized that speciation in the group was partly triggered by the Pleistocene climatic and habitat shifts, as may have been the case in other representatives of the herpetofauna (Raxworthy & Nussbaum, 1996; Vences & Glaw, 1999). Molecular clock estimates in the 16S gene range from ca. 0.3-0.7% pairwise divergence per million years in amphibians and reptiles (e.g. Veith *et al.*, 1998; Caccone *et al.*, 1997; Carranza *et al.*, 2000). Even assuming an accelerated rate of 1% per million years, the youngest split in the *M. domerguei* group (between *M. blommersae* and *M. domerguei*; divergence 4.96%) would be estimated to have occurred five million years before present, and thus at the Miocene/Pliocene boundary. Hence, speciation events among the known species of this group may have taken place well before the Pleistocene, a trend similar to other tropical (Clough & Summers, 2000) and non-tropical vertebrate groups (Avise *et al.*, 1998; Avise, 2000).

Second, morphological differentiation does not appear to be crucial for speciation in these frogs; factors favouring syntopic occurrence of different species are probably ecological (different breeding habitat of *M. blommersae* and *M. sarotra*; possibly different diel activity cycles in *M. domerguei* and *M. kely*) and bioacoustic (different advertisement calls of all species). The low bootstrap support for most relationships within the group does not allow us to formulate a hypothesis for call evolution in the group. The two well-supported groupings, however, are contradictory: *M. kely* and *M. sarotra* (6.20% sequence differentiation) have structurally similar calls, while *M. blommersae* and *M. domerguei* (4.96%) strongly differ in general call structure. More genetic data on other Malagasy anuran groups are necessary to assess the main mechanisms that led to the high number of morphologically similar sibling species (Glaw & Vences, 2000) in this speciose monophyletic radiation (Richards & Moore, 1998; Richards *et al.*, 2000; Vences *et al.*, 2000*a*).

## ACKNOWLEDGEMENTS

We are grateful to Annemarie Ohler and Alain Dubois (MNHN), and Wolfgang Böhme (ZFMK), who allowed examination of specimens held in their care. Nirhy Rabibisoa, Domoina Rakotomalala, Olivier Ramilison, Noro Raminosoa, and Fara Ranaivojaona assisted in the field. The work in Madagascar was made possible by a cooperation between the UADBA and the ZSM. The Malagasy authorities are acknowledged for collection and export permits. The work in Ambatolampy and Manjakatompo was technically supported by the Gesellschaft für Technische Zusammenarbeit; thanks are especially due to Guido Besmer and Jürgen Gräbener. The research was supported by the 'Deutsche Forschungsgemeinschaft' (to F. G.) and the 'Deutscher Akademischer Austauschdienst' (to M. V.). DNA sequencing was financially assisted by the 'Freunde der Zoologischen Staatssammlung e. V.'. The constructive comments of two anonymous referees helped to substantially improve an earlier version of this manuscript.

## REFERENCES

Avise, J. C. (2000). *Phylogeography. The history and formation of species.* Cambridge: Harvard University Press.

Avise, J. C., Walker, D. & Johns, G. C. (1998). Speciation duration and Pleistocene effects on vertebrate phylogeography. *Proc. Roy. Soc. Lond. B* **265**, 1707-1712.

Blommers-Schlösser, R. M. A. (1979). Biosystematics of the Malagasy frogs. 1. Mantellinae (Ranidae). *Beaufortia* **352**, 1-77.

Blommers-Schlösser, R.M.A. & Blanc, Ch.P. (1991). *Amphibiens (première partie). Faune de Madagascar* **75**, 1-384.

Burney, D. A. (1996). Climate and fire ecology as factors in the Quaternary biogeography of Madagascar. In: *Actes du Colloque International Biogéographie de Madagascar.* 49-58. Lourenço, W. R. (ed.). Paris: Société de Biogéographie.

Caccone, A., Milinkovitch, M. C., Sbordoni, V. & Powell, J. R. (1997). Mitochondrial DNA rates and biogeography in European newts (genus *Euproctus*). *Syst. Biol.* **46** , 126-144.

Carranza, S., Arnold, E. N., Mateo, J. A. & López-Jurado, L. F. (2000). Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. *Proc. R. Soc. Lond. B* **267**, 637-649.

Clough, M. & Summers, K. (2000). Phylogenetic systematics and biogeography of the poison frogs: evidence from mitochondrial DNA sequences. *Biol. J. Linn. Soc.* **70**, 515-540.

Glaw, F. & Vences, M. (1994). *A fieldguide to the amphibians and reptiles of Madagascar, second edition.* Köln (Cologne): Vences & Glaw Verlag.

Glaw, F. & Vences, M. (2000). Current counts of species diversity and endemism of Malagasy amphibians and reptiles. In: *Diversité et Endémisme de Madagascar.* 243-248. Lourenço, W.R. & Goodman, S.M. (eds.). Paris: Mémoires de la Société de Biogéographie, Paris.

Glaw, F., Vences, M. & Gossmann, V. (2000). A new species of *Mantidactylus* (subgenus *Guibemantis)* from Madagascar, with a comparative survey of internal femoral gland structure in the genus (Amphibia: Ranidae: Mantellinae). *J. Nat. Hist.* **34**, 1135-1154.

Guibé, J. (1974*a*). Batraciens nouveaux de Madagascar. *Bull. Mus. natn. Hist. nat.*, ser. 3, **145**, 1009-1017.

Guibé, J. (1974*b*). Batraciens nouveaux de Madagascar. *Bull. Mus. natn. Hist. nat.*, ser. 3, **171**, 1169-1192.

Guibé, J. (1975). Batraciens nouveaux de Madagascar. *Bull. Mus. natn. Hist. nat.*, ser. 3, **323**, 1081-1089.

Guibé, J. (1978). Les Batraciens de Madagascar. *Bonn. zool. Monogr.* **11**, 1-140.

Kishino, H. & Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominidea. *J. Mol. Evol.* **29**, 170-179.

Lockhart, P. J., Steel, M. A., Hendy, M. D. & Penny, D. (1994). Recovering evolutionary trees under a more realistic model of sequence evolution. *Mol. Biol. Evol.* **11**, 605-612.

Posada, D. & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.

Raxworthy, C. J. & Nussbaum, R. A. (1996). Montane amphibian and reptile communities in Madagascar. *Conserv. Biol.* **10** , 750-756.

Richards, C. M. & Moore, W. S. (1998). A molecular phylogenetic study of the Old World Treefrogs, family Rhacophoridae. *Herpetol. J.* **8** , 41-46.

Richards, C. M., Nussbaum, R. A. & Raxworthy, C. J. (2000). Phylogenetic relationships within the Madagascan boophids and mantellids as elucidated by mitochondrial ribosomal genes. *Afr. J. Herpetol.* **49**, 23-32.

Swofford, D. L. (2001). *PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods), Version 4.* Sunderland, Massachusetts: Sinauer Associates.

Veith, M., Steinfartz, S., Zardoya, R., Seitz, A. & Meyer, A. (1998). A molecular phylogeny of 'true' salamanders (family Salamandridae) and the evolution of terrestriality of reproductive modes. *J. zool. Syst. evol. Res.* **36**, 7-16.

Vences, M. & Glaw, F. (1999). Variation in *Mantidactylus madecassus* Millot & Guibé, 1950, a little known Malagasy frog, with resurrection of *Mantidactylus pauliani* Guibé, 1974. *Herpetol. J.* **9** , 101-110.

Vences, M., Glaw, F., Kosuch, J., Das, I. & Veith, M. (2000*a*). Polyphyly of *Tomopterna* (Amphibia: Ranidae) based on sequences of the mitochondrial 16S and 12S rRNA genes, and ecological biogeography of Malagasy relict amphibian groups. In: *Diversité et Endémisme de Madagascar*. 229-242. Lourenco, W.R. & Goodman, S.M. (eds.). Paris: Mémoires de la Société de Biogéographie, Paris.

Vences, M., Kosuch, J., Lötters, S., Widmer, A., Köhler, J., Jungfer, K.-H. & Veith, M. (2000*b*). Phylogeny and classification of poison frogs (Amphibia: Dendrobatidae), based on mitochondrial 16S and 12S ribosomal RNA gene sequences. *Mol. Phyl. Evol.* **15,** 34-40.

*Accepted: 14.8.01*