



Two new canyon-dwelling frogs from the arid sandstone Isalo Massif, central-southern Madagascar (Mantellidae, Mantellinae)

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Abstract

We describe two new mantellid frogs of the subfamily Mantellinae from the arid sandstone Isalo Massif, central southern Madagascar. The first - assigned to the genus *Gephyromantis*, subgenus *Phylacomantis* - is a stream frog living in open canyons and associated gallery forest, and is phylogenetically related to *G. corvus* and *G. pseudoasper*. It has a larger maximum SVL than *G. corvus* (43 mm vs. 38 mm in the examined males) and is featured by a mostly contrasted and lighter dorsal pattern. Its advertisement call also differs from that of *G. corvus* in having more numerous and slightly longer notes per call composed of seven to ten sub-units, a clear harmonic structure, and a wider frequency spectrum with a higher dominant frequency. The second new species belongs to the genus *Mantidactylus*, subgenus *Brygoomantis*. It shares morphological and life history similarities with species of the subgenus *Ochthomantis*, in having comparatively expanded fingertips and leading a rupicolous and scansorial life.

Key words: *Gephyromantis*, *Mantidactylus*, Mantellidae, New species, Madagascar, Isalo, Arid environments

Introduction

The taxonomic discovery rate for the rich Malagasy frog fauna has been particularly high in the last decade, due to unprecedented research activity, the use of new study techniques, and the exploration of little studied areas (Köhler et al., 2005).

Within the bulk of more than 230 species (Glaw & Vences, 2006) the highest species diversity is clearly found along the eastern rainforest band (Andreone et al., 2005a), where the speciation process was fast and where the number of species was boosted by the rapid adaptation to the stream habitats (Vences et al., 2002). On the other hand, the biodiversity of the arid western parts of Madagascar has not been systematically surveyed to unveil patterns of amphibian richness and endemism. Studies carried out in the last years have already shown that many undescribed amphibian species are present also in these arid environments. Examples are the discovery of two new *Aglyptodactylus* species (Glaw et al., 1998), and, very recently, of *Scaphiophryne menabensis* Glos, Glaw & Vences (Glos et al., 2005), and even of a new species belonging to a new mantelline genus, *Tsingymantis antitra* Glaw, Hoegg & Vences (Glaw et al., 2006).

The Isalo Massif is a comparatively well-known sandstone area in central-southern Madagascar. It constitutes the main target of many tourists in Madagascar, who are attracted by the beauty of its landscape and by the spectacular canyons. A large portion of the Isalo surface is currently protected as a national park (Anonymous, 2003). The massif has been object of some herpetological works (Glaw & Vences, 1994; Raxworthy &

Nussbaum, 1997) that led at the descriptions of new amphibian species (Busse & Böhme, 1992), or to ecological and conservation considerations regarding two critically endangered species, *Mantella expectata* Busse & Böhme and *Scaphiophryne gottlebei* Busse & Böhme (Andreone et al., 2005b, 2005c, 2006; Mercurio & Andreone, 2005).

Seen this, we decided to carry out a more extended herpetological survey in the Isalo area, also including non protected areas, mainly with the aim of addressing conservation issues regarding the unusual amphibian fauna. Within the framework of our inventory we detected more than 30 reptile species and 21 amphibian species (Mercurio & Andreone, 2006), which stresses the importance of the biodiversity of this area. Among the encountered amphibians, two species turned out to be new to science, while five have been recorded for the first time, resulting in a current total of five potentially locally endemic species. The present paper represents the formal description of a new *Gephyromantis* of the subgenus *Phylacomantis*, and a new *Mantidactylus* of the subgenus *Brygoomantis*.

Materials and methods

Study sites and collecting methods

The sandstone Isalo Massif is located within the Central Ecoregion (Anonymous, 2003). At the closest town, Ranohira, the mean monthly temperature is 25.1 °C, with an absolute minimum of 3.4 °C (June); precipitation is concentrated in the rainy season from late October to February (Anonymous, 1999).

Frogs were searched overnight and located with the aid of hand torches and headlamps, either by opportunistic searches or by the location of calling males. Geographic coordinates were taken using a GPS device. Toponyms follow the indications by local people, and must be therefore seen as unofficial names. The individuals were collected by hand and then euthanised by immersion in chlorobutanol solution, fixed in 4% formalin or in 90° ethanol, and finally stored in 70% ethanol solution. Voucher specimens are currently housed in the Museo Regionale di Scienze Naturali di Torino (MRSN), Forschungsinstitut und Naturmuseum Senckenberg (SMF) and Parc Zoologique et Botanique de Tsimbazaza (PBZT-FAZC). Original field numbers are marked as FAZC (Franco Andreone Zoological Collection).

Morphological measurements

Morphological measurements were made by one of us (VM) with a digital calliper (at the nearest 0.5 mm). Webbing formulae follow Blommers-Schlösser & Blanc (1991), while femoral glands classification follows Glaw et al. (2000). Measurements and counts of granules in glands are taken from internal view of the left gland. The following biometric measurements were taken (according to Andreone et al., 1998): 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 (horizontal tympanum diameter), HAL (hand length), FORL (forelimb length), HIL (hind-limb length), FOL (foot length), FOTL (foot length including tarsus), IMTL, IMTH (length and height of inner metatarsal tubercle), FGL, FGW (length and width of femoral gland), NG (number of granules present in gland), and GD (granules diameter).

Bioacoustic analysis

The advertisement calls were recorded using a digital Sony TCD-D100 recording device with a semi-directional microphone. The sound analysis was carried out with the software Adobe AUDITION 1.5. All the vocalisations were edited with a sampling rate of 44.100 Hz and 16 bits per sample in the mono pattern and in “Waveform” (*.wav) extension. The software was set with the “Hamming” Windowing Function, 1024 bands Fast Fourier Transform (FFT), and a Plot Style of 80–90 dB. Nine traits of the advertisement calls (Wells, 1977) were analysed: notes per call, note duration, intervals between notes, pulses (or sub-units) per note,

pulse (or sub-unit) duration, intervals between pulses (or sub-units), pulse (or sub-units) repetition rate, frequency range, and fundamental and dominant frequency. Definitions of acoustic parameters follow Duellman & Trueb (1986), Littlejohn (2001), and partially Wycherley et al. (2002).

DNA analysis

A fingertip was cut from each collected individual and then stored in 90% ethanol. DNA was extracted from these tissue samples using different standard protocols, and a fragment of the mitochondrial 16S rRNA gene was amplified with the primers 16Sa-L and 16Sb-H of Palumbi et al. (1991). After purification with Qiaquick kits (Qiagen), the fragments were resolved on an automated DNA sequencer (ABI 377 and ABI 3100). Sequences were validated and aligned with the software Sequence Navigator (Applied Biosystems) and deposited in Genbank. The alignment required inclusion of gaps to account for indels in only a few cases in one hypervariable region. Data analysis was carried out with PAUP, version 4b10 (Swofford, 2002).

Results

Gephyromantis (Phylacomantis) azzurrae n. sp.

(Fig. 1)

Diagnosis. A species belonging to the genus *Gephyromantis* (sensu Glaw & Vences, 2006), according to genetic data, and recognizable by the following morphological and natural history characters: (1) medium size (SVL 36–43 mm), (2) webbing between toes present, (3) partly connected lateral metatarsalia, (4) inner and outer metatarsal tubercles present, (5) presence of femoral glands of type 2, (6) presence of a paired subgular vocal sac, (7) finger tips moderately enlarged, (8) nocturnal activity, (9) occurrence in gallery forest habitat of dry central western Madagascar. For comparisons to other *Gephyromantis*, see below.

Holotype. MRSN A5310 (FAZC 12568), adult male, Andriamanero (22°22.40'S; 45°22.71'E; 640 m a.s.l.), Parc National de l'Isalo, Fianarantsoa Faritany, Ranohira Fivondronona, collected by F. Andreone, F. Mattioli, and V. Mercurio on 20 November 2004, fixed in 4% formalin.

Paratypes. MRSN A5309 (FAZC 12567) and A5311 (FAZC 12569), adult males, same locality, collectors and date as the holotype; MRSN A5312 (FAZC 12910), juvenile, collected at Iambahatsy River (22°24.35'S, 45°16.13'E, 689 m a.s.l.), Parc National de l'Isalo, Fianarantsoa Faritany, Ranohira Fivondronona, by V. Mercurio and T.J. Razafindrabe on 15 December 2004, fixed in 4% formalin; MRSN A5313 (FAZC 12951), adult male, collected at Sakamalio (22°26.09'S, 45°15.31'E, 649 m a.s.l.), Parc National de l'Isalo, Fianarantsoa Faritany, Ranohira Fivondronona, by V. Mercurio and T.J. Razafindrabe on 16 December 2004, fixed in 4% formalin; SMF 85859-60 (FAZC 12979-12980) (ex MRSN A5314-5315), adult males, same locality, collectors and date as the MRSN A5313.

Description of the holotype. Specimen in excellent state of preservation missing the left fourth toe (clipped for DNA analysis). Snout-vent length 41 mm, for other measurements see table 1. Body rather stout; head wider than long but not wider than body; snout pointed in dorsal view, rounded in lateral view; nostrils directed posterolaterally, slightly protuberant, nearer to tip of snout than eye; chantus rostralis distinct, straight; tympanum distinct, rounded, 65.5% of eye diameter; tongue ovoid, posteriorly bifid; maxillary and vomerine teeth present; choanae elliptical. Arms slender; fingers without webbing; distinct single subarticular tubercle; finger discs distinctly enlarged, trapezoidal. Hind limbs slender, tibiotarsal articulation reaches between eye and nostrils; lateral metatarsalia partly connected; inner and outer metatarsal tubercles distinct; webbing between toes 1(1), 2i(1.5), 2e(0.5), 3i(2), 3e(1), 4i(2), 4e(2), 5(1). Dorsal skin smooth without evident dorsolateral folds. Ventral skin smooth on throat and chest, granular on belly and limbs. Femoral glands of type 2 fairly distinct from external view; left gland elliptical of 6.3 mm length, 2.0 mm width with about 45

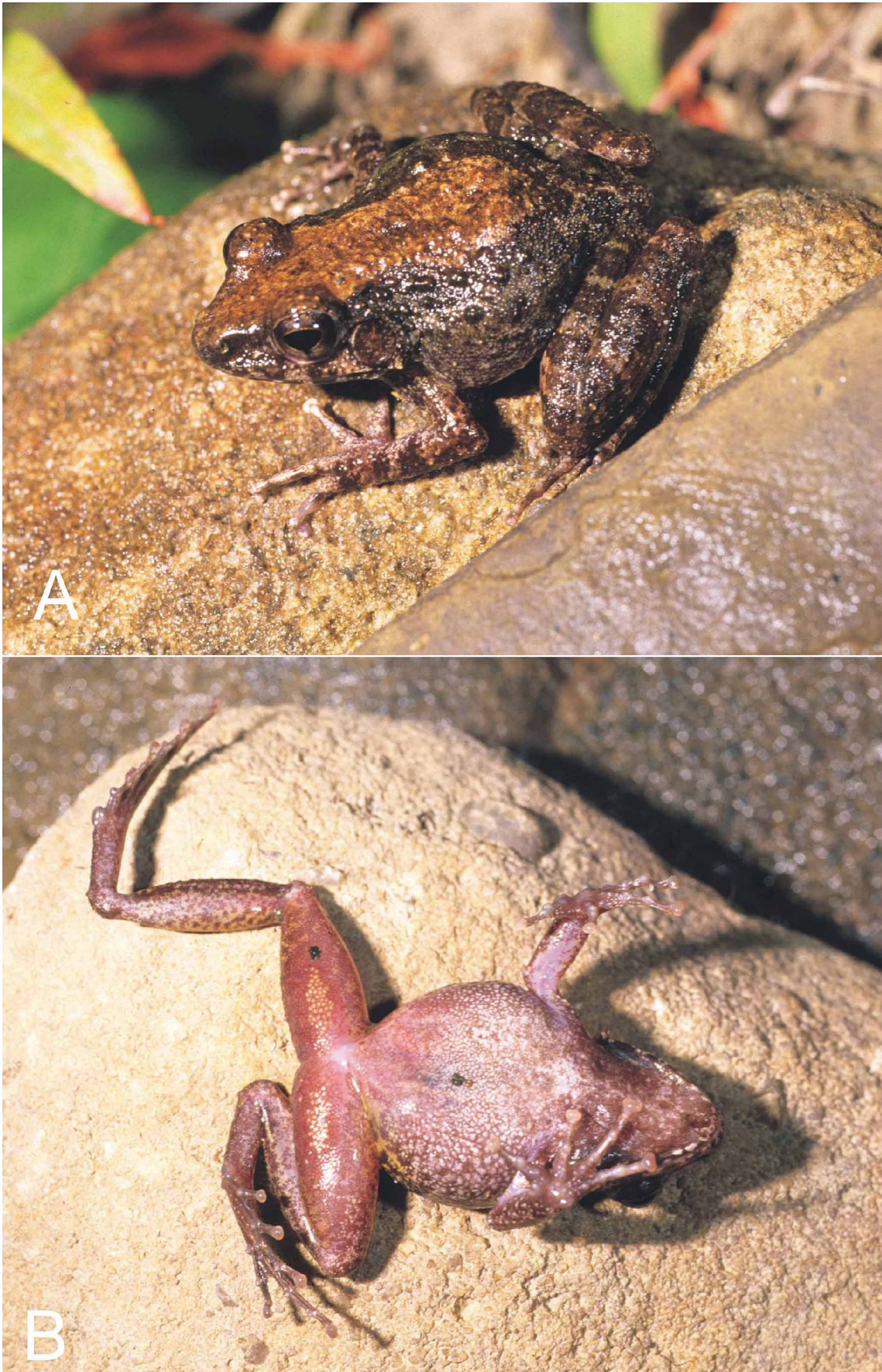


FIGURE 1. *Gephyromantis azzurrae* n.sp. in life: A) dorso-lateral and B) ventral view of the holotype MRSN A5310 (male) from Andriamanero, Isalo Massif.

granules of 0.5 mm diameter. Dorsal colouration brownish-blackish with a dirty light broad vertebral band. Vertebral band with some dark mottlings and bordered by dark. Tympanic area brownish. Faint darker cross-bands present on arms and limbs. Ventrally dark brown mottled by whitish irregular markings, being more abundant along the posterior margins of the arms and extending to axillary region. Brownish folds along lower jaw corresponding to vocal sacs. After one year in preservative the chromatic pattern are similar as in life but strongly faded, especially the lighter vertebral band.

TABLE 1. Morphometric measurements (all in mm) of specimens of *Gephyromantis azzurrae* n.sp.

Catalogue number	Rank	Sex	SVL	HW	HL	TD	ED	END	NSD	NND
MRSN A5310	HT	M	41.1	16.9	13.4	4.0	6.1	3.9	2.5	4.0
MRSN A5309	PT	M	38.5	15.3	12.8	3.7	5.2	4.3	2.2	3.7
MRSN A5311	PT	M	40.2	15.8	14.1	4.1	6.0	4.0	2.7	4.0
MRSN A5312	PT	M	23.3	8.8	8.8	2.5	4.1	2.8	1.4	2.2
MRSN A5313	PT	M	37.2	14.9	12.3	3.3	5.5	4.1	2.3	3.5
SMF 85859	PT	M	42.7	16.4	14.3	3.5	5.4	3.7	2.6	3.9
SMF 85860	PT	M	43.7	16.4	13.5	4.0	5.7	4.0	2.5	3.8

Catalogue number	HAL	FORL	HIL	FOTL	FOL	IMTL	IMTW	FGL	FGW	NG	GD
MRSN A5310	12.1	20.0	41.1	30.0	18.8	2.0	1.1	6.3	2.0	45	0.5
MRSN A5309	11.1	19.9	41.1	26.7	18.9	1.9	1.3	6.5	2.7	38	0.5
MRSN A5311	11.2	19.9	41.0	27.7	19.9	2.1	1.1	6.7	2.7	40	0.6
MRSN A5312	8.8	11.1	24.5	17.7	12.1	1.1	0.5	-	-	-	-
MRSN A5313	11.1	15.6	40.0	26.6	18.9	2.2	0.9	8.2	2.2	29	0.6
SMF 85859	13.4	21.0	41.1	29.9	20.0	1.9	1.1	7.0	3.0	42	0.6
SMF 85860	12.2	21.1	42.3	27.7	20.0	1.9	1.0	7.5	2.7	42	0.5

HT (holotype), PT (paratype), M (male). Other abbreviations are given in the text.

Variation. Paratypes are similar to the holotype in morphology and colouration pattern. The main chromatic differences are the status of dorsal band (brightly visible in MRSN A5309 and A5311 vs. absent in MRSN A5313-5315), and the saturation of ventral colouration (heavily pigmented in MRSN A5309, and SMF 85859-60; pigmented in MRSN A5311; faintly pigmented with a whitish ventral colouration in MRSN A5313). Specimens lacking the vertebral stripe are usually darker with a uniform mottled dorsum without any pattern. Femoral glands are more or less defined and visible from external view in all the specimens. In MRSN A5313 the glands are less visible and reduced with only 29 granules (vs. about 40 in the other specimens) of 0.5 mm diameter of which five are enlarged (0.8 mm) on the posterior margin, while in SMF 85860 they are well visible with well defined margins (7.5 mm length, 2.7 mm width, about 42 granules of 0.5 mm diameter).

Etymology. F. Andreone dedicates this new species to his daughter Kintana Azzurra, with much love and wish of endless happiness.

Natural history. We found individuals of this species inside large canyons with running water in the river bed between stones, as well as in the gallery forest. Calling males were observed at night on leaves in the forest at about 50–150 cm above the ground. Although we do not have quantitative data they appear to be distributed inside the forest at distances of 10–20 m each other. We were also unable to locate tadpoles of *G. azzurrae*: we suspect that they are similar to the ones of *G. pseudoasper* Guibé or *G. corvus* Glaw & Vences. Only two specimens were collected after opportunistic search during the day, while the other specimens by

locating calling males during the night. After the dissection of five specimens the stomach contents contained 12 prey items divided as follows (number of prey item/percentage of the sampling): Hymenoptera, Formicidae (3, 25.0%); Aranea (3, 25.0%); Lepidoptera larvae (2, 16.6%); Coleoptera (2, 16.6%); Brachycera (1, 8.3%), and Heteroptera (1, 8.3%).

Advertisement call. Calling male MRSN A5313 recorded at Sakamalio, Parc National de l'Isalo, on 16 December 2004, c. 20:00, 22°C. The call consisted of a series of 15–21 almost identical complex notes each of which is composed of seven-ten clearly identifiable sub-units arranged in a regular pattern (Fig. 2, table 3). The first two sub-units are longer and, in some cases, separated from each other by a very short time interval (1 ms), and they are followed by 7–8 in amplitude decreasing sub-units (Fig. 3). The frequency spectrum of the call is harmonic. Fundamental frequency is around 1000 Hz followed by five-six well tuned harmonics of which the dominant frequency is about 3000 Hz. Frequency ranges from 800 to 6500 Hz. The temporal structure is periodic with single note of similar amplitude repeated at regular intervals of about 570 ms. We opted for the term “sub-unit” instead of “pulse” to emphasize his unusual complex harmonic structure probably not homologous with that of other *Gephyromantis* and closely related *Mantidactylus* species.

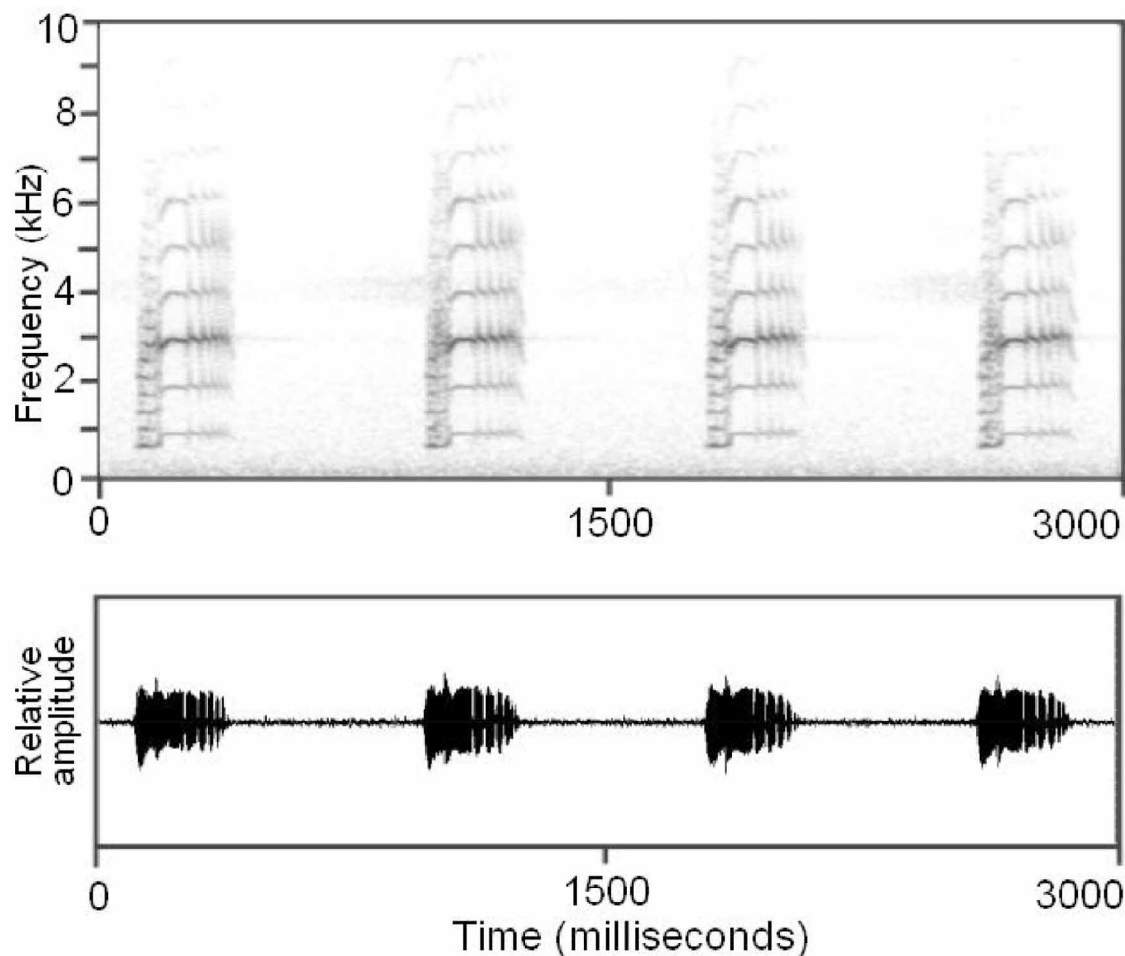


FIGURE 2. Sonagram (above) and oscillogram (below) of the advertisement calls with 4 notes of *Gephyromantis azzurrae* n.sp. from Sakamalio, Isalo Massif.

Morphological comparison with other species. *Gephyromantis azzurrae* bears clear overall morphological similarities to the other two species currently known in the subgenus *Phylacomantis* (*G. pseudoasper* and *G. corvus*), and appears to be genetically closest to these. The new species can be distinguished from *G. pseudoasper* by: (a) darker dorsal and ventral colouration, (b) skin on the back comparatively smoother, (c) larger size (maximum SVL in males in mm: 43 vs. 33–34), (d) advertisement call (15–21 vs. 3 notes per call

and lower dominant frequency, 3000–3200 Hz vs. 3400–5000 Hz). *Gephyromantis azzurrae* differs from the sympatric *G. corvus* by: (a) different dorsal colouration (dark brownish with a dirty vertebral stripe vs. brownish to olive grey with lighter brown patches), (b) different ventral colouration (heavily brown marbled vs. dirty white), (c) comparatively larger eyes and larger tympanum (ratio tympanum/eye diameter 66.7% vs. 60%), (d) body shape (quite stout vs. slender), (e) larger maximum SVL in males in (43 mm vs. 38 mm *corvus*), and (f) advertisement call (see Table 3).

TABLE 2. Morphometric measurements (all in mm) of specimens of *Mantidactylus noralottae* n.sp.

Catalogue number	Rank	Sex	SVL	HW	HL	TD	ED	END	NSD	NND
MRSN A5317	HT	M	34.8	13.5	15.0	4.0	4.9	3.3	2.5	3.7
MRSN A5036	PT	M	35.5	12.9	14.8	4.2	5.2	3.0	2.3	3.7
MRSN A5319	PT	M	33.4	12.2	13.0	3.8	4.0	3.2	1.8	3.6
SMF 85861	PT	M	32.8	13.5	12.8	3.2	5.1	3.4	2.3	3.7
MRSN A5035	PT	F	35.8	13.8	15.6	3.1	4.6	3.9	2.2	4.0
MRSN A5254	PT	F	37.7	13.3	14.5	3.4	5.5	3.4	3.3	4.4
MRSN A5318	PT	F	38.5	15.2	16.3	3.6	5.6	3.2	2.8	4.3
MRSN A5252	PT	F	38.8	14.5	16.6	3.4	5.5	4.4	2.2	4.5
SMF 85862	PT	F	37.8	14.1	14.7	3.3	5.9	3.2	2.8	3.9
SMF 85863	PT	F	35.5	13.3	14.5	3.2	4.4	3.3	3.3	4.4
SMF 85864	PT	F	40.0	15.5	15.5	3.4	5.6	4.4	2.2	3.4

Catalogue number	HAL	FORL	HIL	FOTL	FOL	IMTL	IMTW	FGL	FGW	NG	GD
MRSN A5317	10.0	17.8	36.2	25.9	18.7	2.0	1.2	2.2	2.5	8	0.7
MRSN A5036	11.5	19.8	35.6	29.8	19.3	2.0	1.0	2.0	1.7	8	0.5
MRSN A5319	11.0	18.3	35.6	28.3	18.3	1.6	0.9	2.5	2.0	9	0.6
SMF 85861	9.1	14.9	35.6	28.6	18.3	2.0	1.0	2.0	2.0	4	0.8
MRSN A5035	10.3	18.3	39.1	27.5	19.2	2.0	1.0	-	-	-	-
MRSN A5254	11.1	18.8	39.8	26.7	19.9	2.2	1.0	-	-	-	-
MRSN A5318	11.5	19.8	40.0	30.5	20.5	2.0	1.0	-	-	-	-
MRSN A5252	11.0	18.8	39.8	27.8	19.9	2.2	1.0	-	-	-	-
SMF 85862	9.6	17.5	38.2	29.1	18.8	1.7	0.9	-	-	-	-
SMF 85863	11.1	18.8	37.5	27.0	19.6	2.3	1.0	-	-	-	-
SMF 85864	11.1	20.0	42.2	31.1	21.1	2.2	0.7	-	-	-	-

HT (holotype), PT (paratype), M (male). F (female). Other abbreviations are given in the text.

Mitochondrial differentiation. Accession numbers of newly obtained sequences: MRSN A5309, EF222300; MRSN A5310, EF222301; MRSN A5311, EF222302; SMF 85860, EF222303; MRSN A5312, EF222304; SMF 85859, EF222305). In the mitochondrial 16S rRNA gene, the uncorrected pairwise sequence divergence of *Gephyromantis azzurrae* compared to that of *G. corvus* from Isalo is 7.5%. Concerning the other closely related species, *G. azzurrae* sequence divergences range respectively from 12.4% to 12.9%, for *G. sp. aff. corvus* from Bemaraha, and 15.8%–16.2% for *G. pseudoasper* from Manongarivo (data kindly provided by M. Vences). These divergences are comparatively very high among mantelline species (see Vences *et al.* 2005), and therefore corroborate the species status of *G. azzurrae*.

Conservation. *Gephyromantis azzurrae* is currently known from only three localities in the northern part of the Isalo Massif (Andriamanero, Iamabahatsy, Sakamalio). One of them (Andriamanero) is not included

within the boundaries of the Parc National de l'Isalo. Threats affecting this area include the extensive prairie burning and the alteration due to the excavation of mines for searching sapphires, an activity that has a great potential of long-term persistence and is thus seriously threatening the Isalo biodiversity (Duffy, 2006). Therefore and according to the IUCN (2006) we preliminarily classify *G. azzurrae* as Critically Endangered, following the geographic criteria B2ab(iii). This categorisation could be re-evaluated if all sites of presence will be included within the protected area and/or some other localities will be discovered in the future.

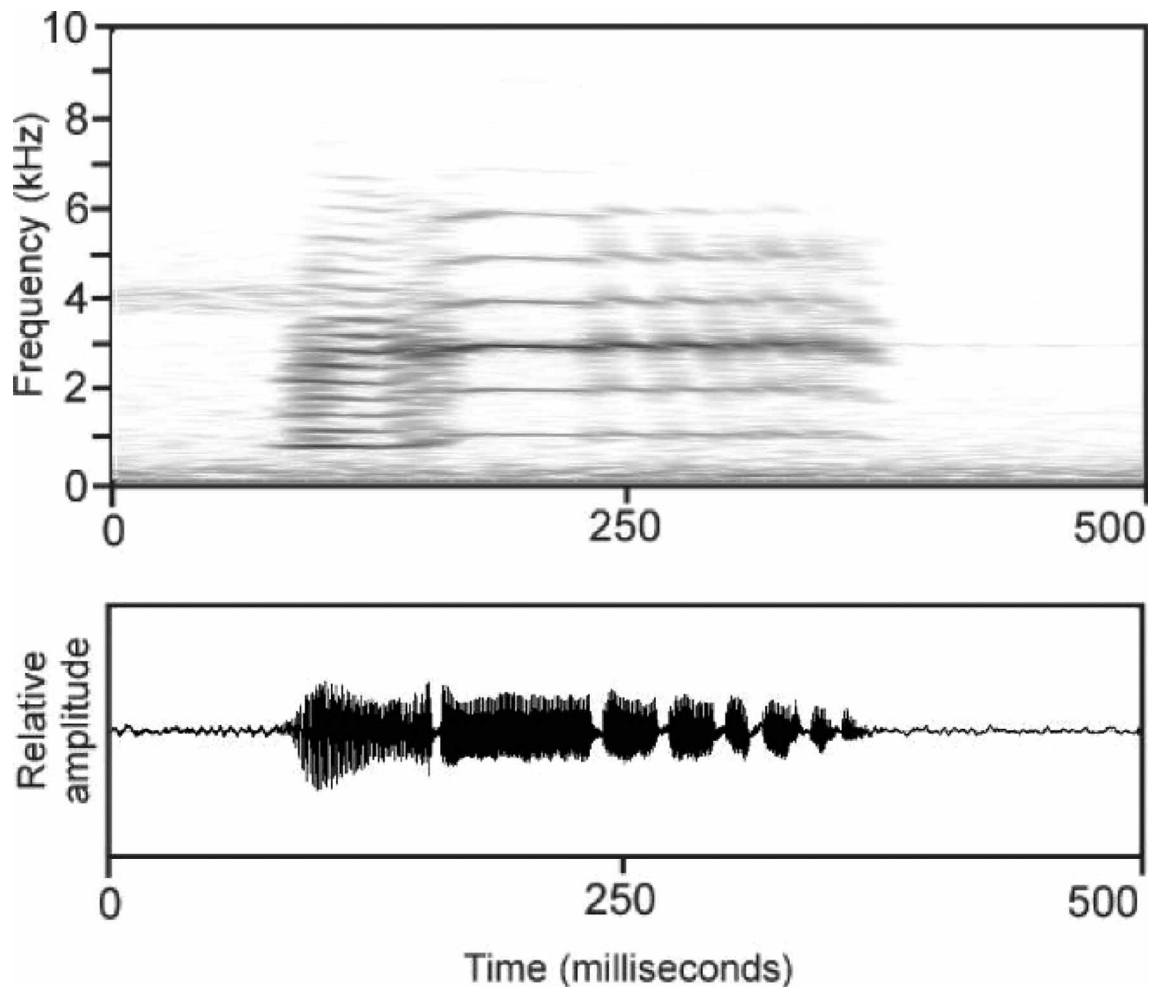


FIGURE 3. Sonogram (above) and oscillogram (below) of one note with eight sub-units of a call of *Gephryromantis azzurrae* n.sp. from Sakamalio, Isalo Massif.

***Mantidactylus (Brygoomantis) noralottae* n. sp.**

(Fig. 4)

Diagnosis. A species attributed to the genus *Mantidactylus* based on the following characters (1) presence of femoral glands of type 3, smaller in females, (2) presence of a single subgular vocal sac, and (3) absence of nuptial pads in males. This species is included in the subgenus *Brygoomantis* due to genetic and bioacoustics evidence. Further morphological characters are: body medium sized; tympanum of males distinctly larger than those of females; tibiotarsal articulation reaches between eye and nostrils; metatarsalia separated; toes webbed; fingertips moderately enlarged; and males with single, subgular vocal sac. Distinction from other species of *Brygoomantis* is mainly based on the isolated distribution and molecular relationships of the new species (see below).

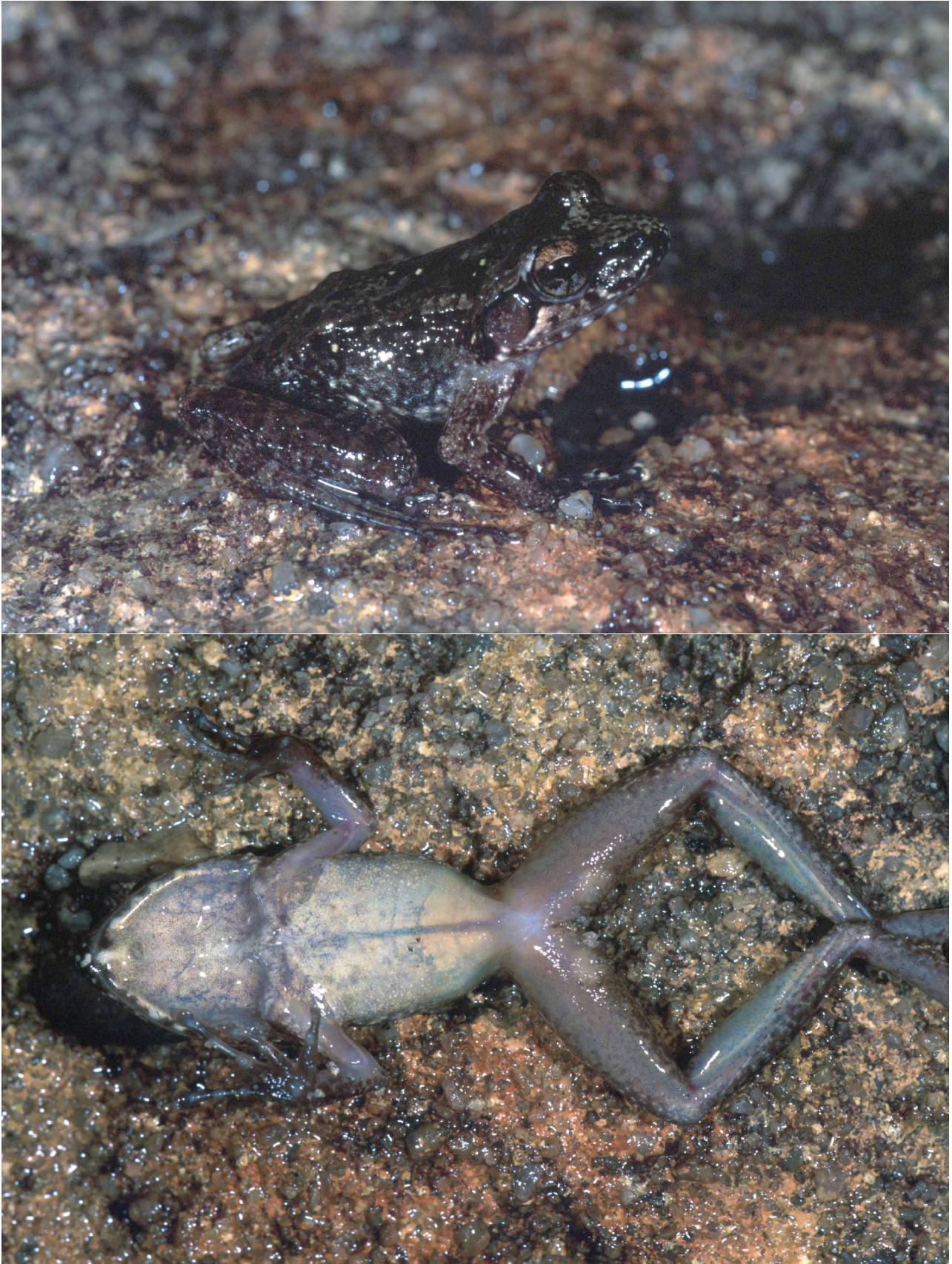


FIGURE 4. *Mantidactylus noralottae* n.sp. in life: A) dorso-lateral and B) ventral view of the paratype MRSN A5319 (male) from Ambovo, Isalo Massif.

Holotype. MRSN A5317 (FAZC 13023), adult male, Ambovo, Parc National de l'Isalo, Fianarantsoa Faritany, Ranohira Fivondronana, 22°30.48'S, 45°21.15'E, 996 m a.s.l, collected by V. Mercurio and T.J. Razafindrabe on 18 December 2004, fixed in 4% formalin.

Paratypes. MRSN A5036 (FAZC 13021) and A5319 (FAZC 13022), adult males; MRSN A5035 (FAZC 13020), A5254 (FAZC 13008) and A5318 (FAZC 13024), adult females; all five specimens with the same locality, date and collectors as the holotype, fixed in 4% formalin; SMF 85861 (ex MRSN A5253 (FAZC 13007)), PBZT-FAZC 12998 (not measured), adult males; SMF 85862-64 (ex MRSN A5255-5257 (FAZC 13011-13013)), MRSN A5252 (FAZC 13005) and PBZT-FAZC 12996 (not measured), adult females; all seven specimens with the same locality, date and collector as the holotype, fixed in 90° ethanol.

Description of the holotype. Male specimen in good state of preservation. Snout-vent length 35 mm, for other measurements see table 2. Body slender; head longer than wide; snout pointed in dorsal view, rounded in lateral view; nostrils directed laterally, protuberant, nearer to tip of snout than eye; canthus rostralis distinct, straight; tympanum distinct, elliptical, 81.6% of eye diameter; supratympanic fold distinct, regularly curved; tongue ovoid, posteriorly bifid; maxillary and vomerine teeth present; choanae elliptical. Arms slender; fingers without webbing; distinct single subarticular tubercle; finger discs distinctly enlarged, elliptical. Hind limbs slender, tibiotarsal articulation reaches between eye and nostrils; lateral metatarsalia separated; inner metatarsal tubercles distinct, outer absent; webbing between toes: 1(1), 2i(1), 2e(0), 3i(1.5), 3e(0.75), 4i(1), 4e(1), 5(0). Dorsal skin smooth without dorsolateral folds. Ventral skin smooth on throat, chest and belly. Femoral glands of type 3 with presence of the "structure B" very distinct from external view, overall appearance of glands very granular, 7.6 mm length and 4.2 mm width; from internal view left gland elliptical of 2.2 mm length, with 8 granules of 0.7 mm diameter. Dorsal colouration brownish softly spotted especially on the canthal region and behind the eyes. Tympanic area brownish bordered by a darker supratympanic fold. Faint darker crossbands present on arms and limbs. Ventrally whitish spotted with irregular brownish markings especially on throat, chest, hands and feet. Vocal sac single, subgular, and slightly distensible. After one year in preservative the chromatic pattern are the same as in life but with faded colours.

TABLE 3. Temporal and spectral parameters in the advertisement calls of *Gephyromantis azzurrae* and *G. corvus* (recorded on 18 December 2004, 19:30). Temporal data are given as range, followed (in parentheses) by mean \pm standard deviation and number of measured elements.

	<i>Gephyromantis azzurrae</i>	<i>Gephyromantis corvus</i>
Locality	Sakamalio	Ambovo
Air temperature	22°C	20°C
Notes per call	15-21 (18 \pm 4.2, n = 2)	10-14 (11.5 \pm 1.7, n = 4)
Note duration (ms)	290-340 (299 \pm 12.8, n = 15)	252-293 (281 \pm 8.0, n = 10)
Intervals between notes	500-740 (573 \pm 92.5, n = 14)	513-716 (580.8 \pm 50, n = 9)
Sub-units per note	7-10 (8.1 \pm 1.1, n = 15)	Non-identifiable
Sub-unit duration (ms)	5-147 (31.5 \pm 35, n = 32)	-
Intervals between sub-units (ms)	1-8 (4.7 \pm 1.67, n = 32)	-
Sub-unit repetition rate (1/s)	7-9 (8 \pm 0.8, n = 7)	-
Frequency (Hz)	800-6500	700-5200
Dominant frequency (Hz)	3000-3200	2400-2700

Variation. The paratypes largely agree with the holotype in morphology and colouration. Chromatic differences lie in: a) presence of a lighter vertebral band; b) extension of darker pigmentation on tympanum; and c) extension and pattern of pigmentation on snout. MRSN A5254 presents a more contrasted dorsal colouration with well defined crossbands on limbs, a light vertebral band, a well defined lighter triangular shaped spot on snout extending to anterior part of eyes, and a darker tympanic area. MRSN A5319 is similar in colour-

ration to the holotype but differs in having a more contrasted light triangle on snout. MRSN A5035 and A5252 are also similar to the holotype but they have a darker tympanic area, as well as SMF 85863 and 85864. SMF 85863 lacks the left foot maybe due to predation. Femoral glands are well defined and visible from external view in all the male specimens, reduced in form of small circular gland of about 1.0 mm diameter composed by a single flattened granule in females.

Etymology. V. Mercurio wishes to dedicate the new species to his wife Nora Lotta Fröhder (now Nora Lotta Mercurio) in recognition of her never-ending support and patience.

Natural history. *Mantidactylus noralottae* is known for the Isalo Massif only. We found several individuals inside a narrow canyon in the initial and gully tracts (Mercurio & Andreone, 2006). Calling males were hanging at night on the almost vertical canyon walls at about 150–200 cm above the bottom or the water surface. Females and males were also found on the canyon bed inside the water pools. As far as known, *M. noralottae* is the only species ascribed to the subgenus *Brygoomantis* with such scansorial habits. They are able to climb almost vertical walls. After the dissection of six specimens to check the stomach contents we found 55 prey items divided as follows (number of prey item/percentage of the sampling): Hymenoptera, Formicidae (33, 60.0%); Coleoptera (16, 29.1%); Diptera (3, 5.4%); Aranea (1, 1.8%); Hymenoptera, undetermined (1, 1.8%); and Orthoptera (1, 1.8%).

TABLE 4. Temporal and spectral parameters in the advertisement calls of *Mantidactylus noralottae*, *M. betsileanus* and *M. sp. aff. ulcerosus*. Temporal data are given as range, followed (in parentheses) by mean \pm standard deviation and number of measured elements. Comparative calls of *M. betsileanus* and *M. sp. aff. ulcerosus* taken from Vences et al. (2006), while further data in parentheses follow Glaw & Vences (1994).

	<i>Mantidactylus noralottae</i>	<i>Mantidactylus betsileanus</i>
Locality	Abovevo, Isalo Massif	Mandraka
Air temperature	20°C	23–24°C
Notes per call	1	1
Note duration (ms)	2400–2900 (2660 \pm 221, n = 5)	2900–3100 (3053 \pm 82.1, n = 4)
Intervals between notes	5000–7000 (6000 \pm 1414, n = 2)	11900–13000 (12660 \pm 577.1, n = 3)
Pulses per note	90–100 (96.6 \pm 1.4, n = 5)	163–178 (172.5 \pm 6.5, n = 4)
Pulse duration (ms)	10–26 (15.8 \pm 3.3, n = 25)	6–8 (6.8 \pm 0.8, n = 15)
Intervals between pulses (ms)	8–16 (12.5 \pm 2.2, n = 17)	8–10 (9.1 \pm 0.9, n = 10)
Pulse repetition rate (1/s)	41–45 (43 \pm 1.8, n = 5)	55–64 (58.6 \pm 4.7, n = 3)
Frequency (Hz)	900–3000	400–5000
Dominant frequency (Hz)	1300–1500	1400–1500

	<i>Mantidactylus sp. aff. ulcerosus</i>
Locality	Ranohira, Isalo Massif
Air temperature	23.4°C
Notes per call	8–(14)
Note duration (ms)	113–142 (126.3 \pm 8.8, n = 8)
Intervals between notes	130–170 (152.1 \pm 8.4, n = 7)
Pulses per note	22–26 (24.8 \pm 1.3, n = 8)
Pulse duration (ms)	1–10 (4.5 \pm 2.3, n = 35)
Intervals between pulses (ms)	None
Pulse repetition rate (1/s)	96–102 (99 \pm 3, n = 2)
Frequency (Hz)	700–4500
Dominant frequency (Hz)	1000–1200

Advertisement call. Calling male MRSN A5319 recorded at Ambovo, Parc National de l'Isalo (on 18 December 2004, 20:00, 20°C). The call consisted of a single long note (2.6 sec) composed of a train of about 100 short pulses (Fig. 5, table 4). The pulse train originates during expiration and is associated with a strong vibration of the body wall. Pulses are poorly toned and harmonics are not clearly visible in the spectrogram. Fundamental frequency is around 1000 Hz while dominant frequency is about 1400 Hz. Frequency ranges from 900 to 3000 Hz. The calls are emitted after rather long time intervals (6 sec).

Morphological comparison with other species. Based on the basis of genetic and bioacoustics evidences *Mantidactylus noralottae* has been placed in the subgenus *Brygoomantis*. However, like the other species belonging to this subgenus superficial similarities are shared with the species belonging to subgenus *Ochthomantis*. Convincing synapomorphies on adult morphology able to clearly cut species belonging to both subgenera are currently unknown (Glaw & Vences, 2006). In particular, at the Isalo Massif *M. noralottae* lives in sympatry with *M. sp. aff. ulcerosus* (Boettger) and in syntopy with *M. cf. femoralis* (Boulenger). Adults of *M. noralottae* can be confused with juveniles of *M. cf. femoralis* and, at a lesser extent, with adult specimens of *M. sp. aff. ulcerosus*. *Mantidactylus noralottae* can be distinguished from *M. sp. aff. ulcerosus* by: (a) different dorsal colouration (brownish softly spotted vs. uniformly light brown to greyish sometimes with darker patches); (b) dorsal skin (smooth vs. granular); (c) general overall appearance (slender vs. stout); (d) advertisement call (single prolonged note vs. 8–14 notes). It can be distinguished from the other *Brygoomantis* species by the following combinations of characters: body dimension (smaller in *M. alutus* (Peracca), *M. biporus* (Boulenger), *M. tricinatus* (Guibé), and larger in *M. ambohimitombi* Boulenger); by dorsal colouration and presence of vomerine teeth (*M. madecassus* Millot & Guibé and *M. pauliani* Guibé); by dorsal skin texture and hind limb length (skin granular in both *M. betsileanus* and *M. curtus*, hind limbs comparatively longer in the first and shorter in the second); and by different advertisement calls (from the recently resurrected taxa *M. bellyi* Mocquard and *M. bourgati* Guibé). Convincing differences in call parameters between *M. noralottae* and *M. betsileanus* are still in need of identification, largely depending on an upcoming taxonomic revision of the subgenus *Brygoomantis*. *Mantidactylus noralottae* can be distinguished from the syntopic *M. cf. femoralis* by: (a) different dorsal colouration (lighter without black stripes and yellow spot on flanks vs. darker with presence of black stripes and yellow spot on flanks), (b) different ventral colouration (slightly marbled on chest, belly dirty without black lines on throat vs. heavily marbled, belly spotted, black lines on throat often present), (c) hind limbs length (tibiotarsal articulation reaches between eyes and nostrils vs. tip of snout or beyond); (d) body size (34.1 mm, males, and 37.7 mm, females vs. 37.8 mm, males, and 44.7 mm, females) and, (e) body shape (slender vs. quite stout). *Mantidactylus noralottae* can be distinguished from the other species belonging to the *Ochthomantis* subgenus (*M. ambreensis* Mocquard, *M. majori* Boulenger, *M. mocquardi* Angel and *M. zolitschka* Glaw & Vences) by: (a) dorsal colouration uniformly brownish more or less spotted, (b) dorsal skin smooth, (c) smaller body size (male SVL < 40 mm vs. > 40 mm except for *M. ambreensis*) and (d) comparatively enlarged inner metatarsal tubercle, (e) advertisement call.

Mitochondrial differentiation. Accession numbers of newly obtained sequences: MRSN A5252, EF222306; SMF 85861, EF222307; MRSN A5254, EF222308; SMF 85862, EF222309; SMF 85863, EF222310; SMF 85863, EF222311. The uncorrected pairwise DNA divergence of the *Mantidactylus noralottae* 16S rRNA gene sequence compared to that of *M. betsileanus* from Mandraka (East Madagascar) is 3.6%, while the difference with *M. ulcerosus* from Berara (NW Madagascar) corresponds to 11.5%–11.9% (M. Vences, pers. comm.). This divergence fully supports the specific status of *M. noralottae*.

Conservation. *Mantidactylus noralottae*, similarly to *Gephyromantis azzurrae*, appears to be a localized species occurring only in the northern part of the Isalo Massif. Taken into consideration that it is known from a single locality within the Parc National de l'Isalo we cannot say too much about the impending threats. However, we preliminarily classify *M. noralottae* as Critically Endangered, following the geographic criteria, B2ab(iii). As for *G. azzurrae* we consider that this categorisation could be changed if some other localities (outside the Isalo massif) will be discovered in the future.

Available names. According to Blommers-Schlösser & Blanc (1991), several available names in the sub-genus *Brygoomantis* are to be considered as junior synonyms of valid species or dubious names. *Mantidactylus noralottae* differs genetically from all sequenced *Brygoomantis* species belonging to the *M. betsileanus* group (M. Vences, pers. comm.), thus representing a likely vicariant western species. Taxa known from western localities (potentially conspecific with *M. noralottae*) are *Rhacophorus fumigatus* Mocquard (type locality "Madagascar côte ouest") and *Mantidactylus brunneus* Ahl (type locality "Nord-West-Madagascar") both currently considered as synonyms of *M. betsileanus*, and *Mantidactylus tripunctatus* Angel (type localities "Pic St. Louis, province de Fort-Dauphin" and "Befotaka, province de Farafangana"), considered as *nomen dubium* (Glaw & Vences, 1994; 1999). According to the original description of the holotype of *M. tripunctatus* the finger discs are very small and smaller than those of toes; the tibiotarsal articulation reaches the tip of snout or between tip of snout and eyes; SVL of largest syntype is 30 mm. *M. noralottae* can be unequivocally distinguished from *M. tripunctatus* by the combination of comparatively larger finger discs, shorter hind limbs, and larger body size.

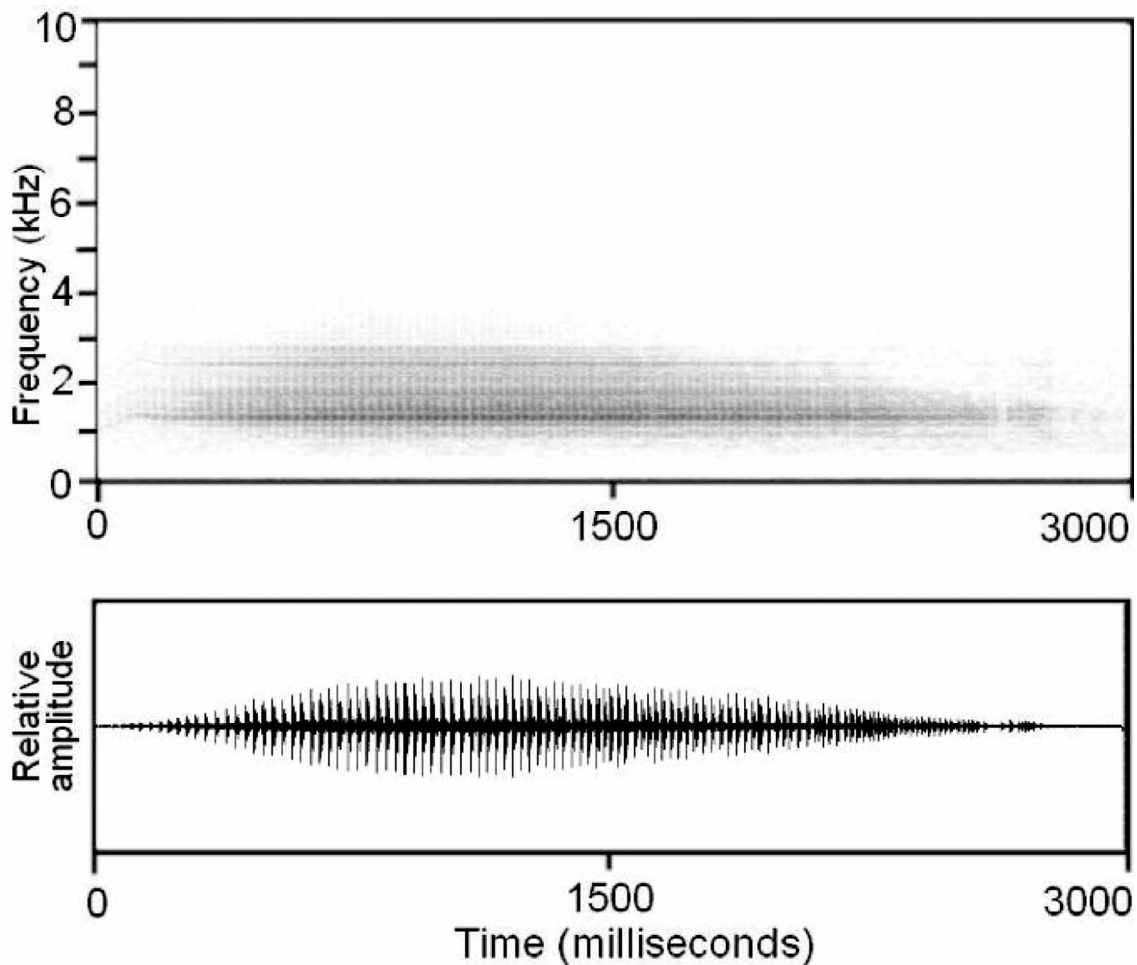


FIGURE 5. Sonogram (above) and oscillogram (below) of the advertisement calls of *Mantidactylus noralottae* n.sp. from Ambovo, Isalo Massif.

Discussion

Despite its apparently hostile appearance and aridity, the Isalo Massif harbours a diverse frog fauna. Our survey works, pooled with scattered data formerly collected by other researchers, gave a total of at least 21 frog species (Mercurio & Andreone, 2006). This is a comparatively high number, especially if we consider that

there are apparently many regionally endemic and threatened species, such as *Scaphiophryne gottlebei*, *Mantella expectata*, *Gephyromantis corvus*, *G. azzurrae*, and *Mantidactylus noralottae*. Beside this list, several other species may represent vicariant entities still to describe, such as a *Mantidactylus*, subgenus *Brygoomantis*, and a *Boophis* Tschudi of the *goudoti* group. It is likely that the amphibian fauna of Isalo is not yet completely known, and we suspect that other unknown species will be discovered in the near future.

All this stresses that the Isalo Massif is indeed peculiar in terms of biogeography, and likely acted as refuge for several frog species during the climatic changes of the Pleistocene-Holocene (Burney, 1997) and previous periods. The presence of narrow canyons within the ruiniform sandstone formations also allowed the persistence of evergreen gallery forests although the shift in more dry climatic conditions, acting as refuges for humidity dependent species. This view is supported by the presence in this arid area of typical rainforests species like *Mantidactylus* cf. *lugubris* Duméril, *M.* cf. *femorialis*, *Boophis* cf. *goudoti* Tschudi, and *B. luteus* (Boulenger) (Raxworthy & Nussbaum, 1997). The isolation of the humid forest blocks within a dry savannah habitat likely favoured the differentiation of new endemic species, like the ones we have herein described. However, the relatively high genetic divergences of the new species to their respective closest relatives indicate that these potential vicariant processes may occur during the Pleistocene, or even the Pliocene.

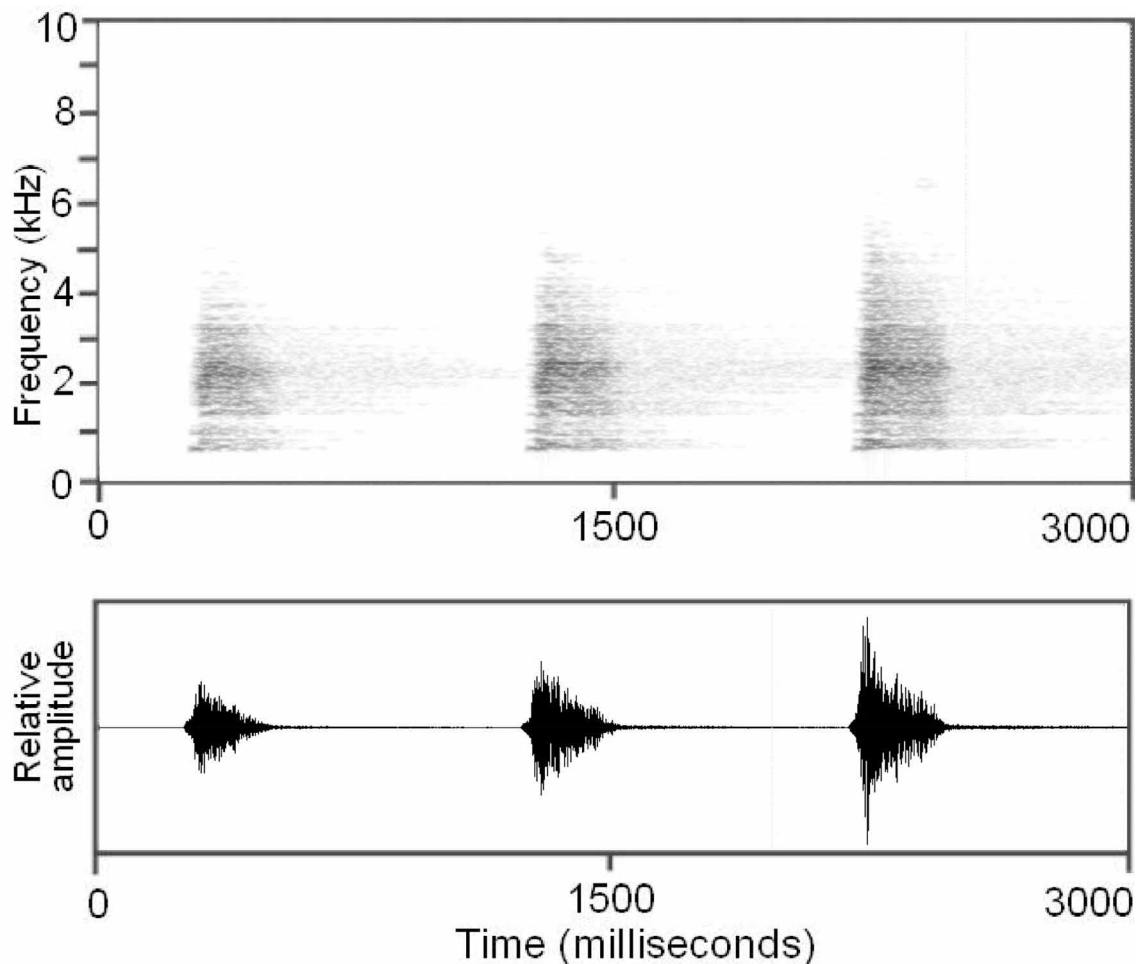


FIGURE 6. Sonagram (above) and oscillogram (below) of the advertisement calls with 3 notes of *Gephyromantis corvus* from Ambovo, Isalo Massif.

The two newly described species are surprising in many ways. *Gephyromantis azzurrae* is genetically and morphologically close to *G. corvus*. The syntopic occurrence of these two sister species is remarkable. So far, *G. corvus* appears more typical of close and gully canyons, while *G. azzurrae* prefers more open valleys, with abundant riverine vegetation. The advertisement call of *G. azzurrae* consists in the emission of a consecutive

series of complex harmonic notes with a relatively low repetition rate. This differs in some relevant temporal and spectral parameters from the advertisement call of the closely related *G. corvus*. On the basis of the available data the call of *G. azzurrae* differs from them of *G. corvus* in the temporal structure in having more notes per call composed of clearly identifiable sub-units and longer notes per call, while in spectral structure for a wider frequency spectrum, a higher dominant frequency (see Table 3), and a much more harmonic structure. The advertisement call of *G. corvus* (Figs. 5, 6) was reported by Glaw & Vences (1994) to show a frequency range from 2200 to 4500 Hz, with a dominant frequency of about 3500 Hz, and a note repetition rate of 1.1/s. Our recordings for this species show a lower dominant frequency (2400–2600 Hz), a wider frequency range (700 to 5400 Hz) and similar note repetition rate of 1.1 to 1.25/s. Since the temporal and spectral parameters are still comparable, these differences may be due to different recording distances, with our recording probably obtained from further away, or to different recording equipment used.

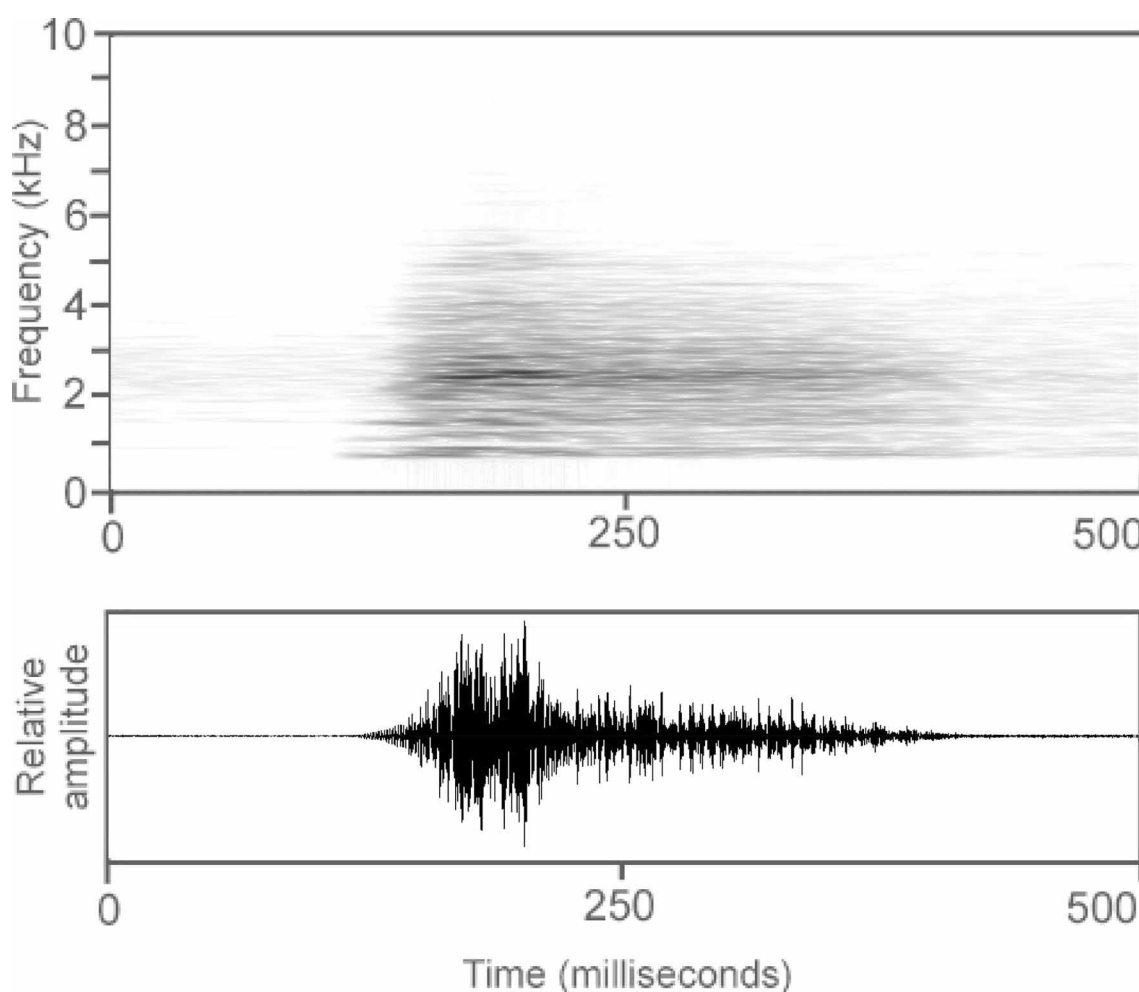


FIGURE 7. Sonagram (above) and oscillogram (below) of one note of a call of *Gephyromantis corvus* from Ambovo, Isalo Massif. Note the absence of well identifiable sub-units.

Mantidactylus noralottae represents the first known western species of the widespread *M. betsileanus* group. The discovery of these probably Isalo endemic species highlights the role of the massif as centre of endemism through the occurrences of allopatric speciation. As for *Gephyromantis corvus* and *Scaphiophryne gottlebei*, *M. noralottae* seems to be ecologically adapted to the canyon habitat. The unusually enlarged fingertips for a species belonging to *Brygoomantis* subgenus are likely a specialisation apt to facilitate a scansorial life style.

The presence of at least five possibly endemic frog species (*Mantella expectata*, *Scaphiophryne gottlebei*, *Gephyromantis azzurrae*, *G. corvus*, and *Mantidactylus noralottae*) indicates that the Isalo Massif is indeed a

very crucial center of endemism and crucial for the amphibian conservation. All these species should be carefully monitored in the next years, and should become object of a careful conservation planning.

Acknowledgments

The Isalo survey was done in collaboration with the Parc Botanique et Zoologique de Tsimbazaza (Antananarivo) and the Université d'Antananarivo. We thank G. Aprea, F. Mattioli, J.E. Randrianirina, and T.J. Razafindrabe for their help in the field. G. Hantke for the kind help provided in identification and count of the stomach contents. V. Mercurio wishes to thank R. Marquez and J. Wycherley for the useful assistance with call analysis. M. Vences and A. Crottini kindly assisted with the genetical analysis. The fieldwork was financially supported by DAPTF, Air Madagascar, Acquario di Genova, Nando Peretti Foundation, and Gondwana Conservation and Research.

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