A new cryptic frog of the genus Boophis from the north-western rainforests of Madagascar

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Abstract.—We describe a new species of arboreal frog of the Boophis mandraka group (Anura: Mantellidae) from Manongarivo Special Reserve in north-western Madagascar. This new species is morphologically similar to the other species of this group: B. liami, B. mandraka, and B. solomaso. It differs by its advertisement calls which are a regular series of frequency-modulated whistling notes of 61-82 milliseconds duration. Analysis of a fragment of the mitochondrial 16S rRNA gene grouped these four species in a single clade but was unable to assess relationships within this clade. Pairwise genetic divergence of the new species to related taxa in this gene fragment was 5.3-8.3%, indicating a long isolated evolutionary history.

Key words.—Amphibia, Mantellidae, Boophis mandraka group, new species, Sambirano region, systematics, molecular phylogeny.

The faunistic and floristic uniqueness of north-western Madagascar is well known since the work of Perrier de la Bathie (1927). While the southern and central parts of the island have a relatively clear division between dry western areas and humid eastern rainforests, in the northern part the rainforests reach the western coast. Although highly fragmented due to human activities at present, it can be assumed that this east-west rainforest connection was continuous into the Holocene (Vences et al. 2003a). Wells (2003) hypothesizes that the northwestern (Sambirano) monsoon forest originated in the Late Miocene or Pliocene, whereas the eastern rainforests had already emerged in the Eocene or Oligocene.

Early attempts at defining biogeographic regions in Madagascar named these north-western lowland rainforests as the Sambirano region, and emphasized their high degree of endemism (e.g., Angel 1942). More recent works on amphibians and reptiles indicated a more complex pattern, with species of the mid-altitudes occurring in both the north-western and eastern rainforests (e.g., Rakotomalala et al. 2001). However, there is a high degree of endemism at higher altitudes, especially on the Tsaratanana massif, the highest mountain of Madagascar reaching 2876 m above sea level (Vences et al. 2003a). The low altitude forests in north-western Madagascar are also characterized by a unique amphibian fauna (Andreone et al. 2003), although some of the typical species occur in the north or north-east as well (Vences et al. 2003a).

During a recent survey of the low- and mid-altitudes of the Manongarivo massif in north-western Madagascar we discovered several taxa that had not been recorded from this area before, such as a yet undetermined leaf-axil breeding Mantidactylus of the subgenus Pandanusicola. We also collected frogs that represent several species new to science. Herein, we describe one new low-altitude species from this site, a member of the Boophis mandraka group of mantellid treefrogs that had not previously been recorded from north-west Madagascar.
MATERIALS AND METHODS

Frogs were captured by locating calling males during the night. They were killed using chlorobutanol solution, fixed either in 5% formalin or 95% ethanol, and preserved in 70% ethanol. Voucher specimens were deposited in the herpetological collections of the Université d’Antananarivo, Departement de Biologie Animale (UADBA), Zoologische Staats-sammlung München (ZSM), and Zoological Museum Amsterdam (ZMA).

Morphometric measurements were taken by the senior author to the nearest 0.1 mm using vernier calipers. Webbing formula is used according to Blommers-Schlösser (1979a). The following abbreviations are used in this work:

- SVL—Snout-vent length
- HW—maximum head width
- HL—head length from tip of snout to posterior edge of snout opening
- TD—horizontal tympanum diameter
- ED—horizontal eye diameter
- END—distance between anterior edge of eye and nostril
- NSD—distance between nostril and tip of snout
- NND—distance between both nostrils
- FORL—forelimb length, from limb insertion to tip of longest finger
- HAL—hand length, to the tip of the longest finger
- HIL—hindlimb length, from the cloaca to the tip of the longest toe
- TIL—tibia length
- FOTL—foot length including tarsus
- FOL—foot length

Muscle tissue samples were taken from freshly killed adults and larval specimens in the field and preserved in pure ethanol. DNA was extracted and a fragment of the mitochondrial 16S rRNA gene amplified and sequenced using the primers 16SA-L and 16SB-H of Palumbi et al. (1991) and followed standard procedures (e.g., Vences et al. 2003b). The sequences were aligned with those of other Boophis species, as obtained by Vences et al. (2002) and Vallan et al. (2003) and available from Genbank.

Sequence alignment was carried out using the software Sequence Navigator (Applied Biosystems). Because several regions of the alignment required the inclusion of gaps to account for multiple indels and could therefore not be reliably aligned, we performed analyses after excluding these hypervariable sections. Phylogenetic analysis was carried out using PAUP, version 4b10 (Swofford 2002). We performed unweighted maximum parsimony heuristic searches, with tree-bisection reconnection branch swapping, and random addition sequence with 100 replicates. Robustness of nodes was tested by full heuristic bootstrapping, with 2000 pseudoreplicates (and 10 random addition sequence replicates) under maximum parsimony.

Advertisement calls were recorded with different tape recorders and microphones, and were
analysed with the program CoolEdit (Syntrillium Corp.) on a PC. Temporal measurements in the call description are given as range, with mean ± standard deviation.

RESULTS

The *Boophis rappiodes* species group as initially defined by Blommers-Schlösser (1979b) has turned out to be paraphyletic based on molecular studies (Vences et al. 2002). Although the species *B. liami*, *B. mandraka* and *B. solomaso* share with the other species of that group their general external appearance, small size and translucent ventral skin, they seem to form a separate clade within the stream-breeding *Boophis* lineage. Consequently, and anticipating a more comprehensive revision of mantellid classification, we recognize a *Boophis mandraka* group, that contains the three species mentioned above and a new species from north-western Madagascar that we here describe.

SYSTEMATICS

*Boophis sambirano* sp. nov.

Figs. 1-2.

Holotype.—Adult male, ZSM 811/2003 (original field number FG/MV 2002.708), collected by F. Glaw, M. Vences and R.-D. Randrianiaina on 31 January 2003 at a small settlement called “Camp Norbert” by our guides, 13° 56’ 53” S, 48° 27’ 28” E, ca. 280 m above sea level, Manongarivo Special Reserve, northwestern Madagascar.


Diagnosis.—The new species is assigned to the genus *Boophis* based on the presence of an intercalary element between ultimate and penultimate phalanges of fingers and toes (as assessed by external examination), presence of (poorly defined) nuptial pads in males, molecular phylogenetic relationships (see below), and phenetic similarity to other species of the *B. mandraka* group. *Boophis sambirano* is characterized by small body size (17-23 mm SVL), greenish dorsal colouration and translucent ventral colouration. It shares these character states with species of the *B. mandraka* group. Within the *B. rappiodes* group, it is distinguished from *B. viridis* by smaller size (SVL of adult males 17-23 vs. 29-31 mm), from *B. erythrodactylus*, *B. tasymenta*, *B. rappiodes* and *B. bottae* by the absence of red or reddish spots on the dorsum and by having the nostrils nearer to eye than to the tip of the snout (vs. nearer to the tip of the snout in the other species).

Molecular data corroborate that *B. sambirano* falls within the *B. mandraka* group (Fig. 3). Within this group it differs from *B. mandraka* by the lack of silvery reticulations on the iris. Furthermore, the advertisement calls of *B. sambirano* are distinct. Compared to *B. liami*, the note duration is shorter (61-82 ms vs. 139-232 ms) and note interval duration is longer (974-1289 ms vs. 168-342). Compared to *B. solomaso*, note duration is much shorter (61-82 ms vs. 422-571 ms) and note interval duration is longer (974-1289 ms vs. 229-274 ms). Compared to *B. mandraka*, the calls are emitted much more regularly, notes are of more uniform duration and structure (irregular series of
chirps of variable duration in *B. mandraka*), and the notes are distinctly frequency-modulated (not frequency-modulated in *B. mandraka*).

**Description of the holotype.**—An adult male (found emitting advertisement calls). It is in good state of preservation. A small amount of muscle tissue from left thigh removed for molecular analysis. For measurements see Table 1. Body slender; head wider than long, wider than body; snout rounded in dorsal and lateral view; nostrils directed dorsolaterally, slightly protuberant, closer to eye than to tip of snout; canthus rostralis indistinct, slightly concave; loreal region very slightly concave; tympanum distinct, small, its diameter is 39% of eye diameter; supratympanic fold indistinct and slightly curved; tongue ovoid, distinctly bifid posteriorly; vomerine teeth as one distinct oblong group posterolateral of each choana, the two groups almost in median contact; choanae small, rounded. Forelimbs slender; subarticular tubercles single; inner and outer metacarpal tubercles not recognizable. Webbing among fingers: 1(traces) 2(traces) 3i(traces) 3e(1.5) 4i(1); relative finger length 1<2<4<3; finger disks moderately enlarged; pigmentless faint nuptial pads on inner side of first finger. Legs slender, when legs are adpressed along body, the tibiotarsal articulation reaches beyond snout tip; lateral metatarsalia separated; inner metatarsal tubercle small, outer metatarsal tubercle absent; webbing formula of the foot 1(0), 2i(1); 2e(0); 3i(1); 3e(0.5); 4i/e(1.5), 5(0); relative toe length 1<2<3<5<4. Skin on the dorsum smooth; ventral skin smooth on throat and chest, slightly granular on belly.

In preservative, yellowish white with irregular dense brown marbling on dorsum and limbs. Large brown patches above each eye. A distinct white line, bordered by brown, runs from posterior edge of eye, dorsally over eye and nostrils, to the tip of snout. Uniformly yellowish white on ventral side.

**Colour in life.**—Green with yellowish tones and brown marbling on dorsum. The patches above the eyes were orange, the line between eye and snout tip was yellow. The iris was reddish brown centrally, fading to beige externally. The iris periphery was blue. Ventrally greenish translucent, the white inner organs visible through ventral skin (Fig. 2).

**Variation.**—The three UADBA paratypes were not available for examination. Morphology of the examined paratypes agrees with that of the holotype (Table 1). Dorsum always with a variable extent of brown markings that in some specimens (e.g., ZMA 19569) can be very dense, almost covering the entire dorsum. Light line from eye to tip of snout not present in all specimens.
Etymology.—The specific epithet refers to the river Sambirano that gave name to the whole region in north-western Madagascar where the type locality of the new species is located. The name is used as an invariable noun in apposition to the generic name.

Molecular phylogenetic relationships.—After exclusion of 48 hypervariable sites the data set contained 491 characters of which 350 were constant and 84 were parsimony-informative. The maximum parsimony analysis yielded a single most parsimonious tree (Fig. 3) with a length of 299 steps and a consistency index of 0.553. Boophis sambirano was sister to B. solomaso. The B. mandraka group with B. liami, B. sambirano and B. solomaso was a clade, although it was not supported by a relevant bootstrap value (< 50%). The B. rappiodes group (here included: B. bottae, B. erythrodactylus, B. rappiodes, B. tasymena) was a highly supported clade (bootstrap value: 100%) and did not appear to be related to the B. mandraka group. Uncorrected pairwise divergences in the DNA fragment sequenced were 8.3% (41

Table 1. Morphological measurements (mm) of holotype and six paratypes of Boophis sambirano sp.nov. (all males). For abbreviations, see Materials and Methods; additional abbreviations used: HT—holotype, PT—paratype, RHL—relative hindlimb length. RHL is measured as the position of the tibiotarsal articulation when hindlimb is adpressed along body: a—between eye and nostril; b—nostril; c—snout tip; d—beyond snout tip.

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Figure 3. Maximum parsimony phylogram of species in the Boophis mandraka group (B. liami, B. mandraka, B. sambirano, B. solomaso) and representatives of other Boophis groups. The tree was obtained after exclusion of all hypervariable regions of uncertain alignment that included gaps. Numbers are support values from bootstrap analyses (2000 replicates) in percent (only shown if > 50%). The Boophis mandraka group is shown in a grey box.
substitutions and indels) between *B. sambirano* and *B. liami*, 5.3% (30 substitutions and indels) between *B. sambirano* and *B. mandraka*, and 6.8% (32 substitutions and indels) between *B. sambirano* and *B. solomaso*.

**Natural history.**—Numerous male specimens were found calling in a degraded area near a small human settlement, not far from areas of relatively undisturbed rainforest. Specimens were calling 5-20 m from the water, along a fast-flowing stream of ca. 3 m width, flowing over sandy and rocky bottoms. Calling males were sitting 2-4 m high in the vegetation on shrubs and trees.

**Distribution.**—So far the new species is reliably known only from the type locality, Manongarivo. A single specimen collected at Antsahamanara campsite (Manarikoba forest, in the Tsaratanana massif) by J. Randrianirina, F. Andreone, F. Mattioli and M. Vences in 2001 may also belong to this species, but its identity remains to be clarified by a future study. Specimens assigned to *B. mandraka* from various localities on the Masoala Peninsula are not conspecific with *B. sambirano* according to unpublished genetic data (F. Andreone, pers. comm.).

Advertisement call. Recorded on 31 January 2003, ca. 18:45 h, at an air temperature of 25.8 °C. The call is a series of melodious whistling notes (Fig. 4) that is repeated at regular intervals. Three calls contained 12, 16 and 20 notes each. Note duration is 61-82 ms (71 ± 7 ms; N = 10), duration of the interval between notes is 974-1289 ms (1161 ± 107 ms; N = 10). Notes are frequency modulated; they start with a frequency band at 3900-4100 Hz and go up to 4400-4800 Hz.

![Figure 4. Sonagram and oscillogram of one note of the advertisement call of Boophis sambirano sp. nov., recorded at Manongarivo on 31 January 2003, recorded at an air temperature of 25.8 °C.](image-url)
DISCUSSION

The discovery of *B. sambirano* corroborates that many species of widespread amphibians, in Madagascar, are indeed complexes of cryptic species. After the description of two species close to *Boophis mandraka* by Vallan et al. (2003), occurring geographically close to *B. mandraka* in central-eastern Madagascar, it was to be expected that records of *B. mandraka* from northeastern Madagascar, i.e., Marojejy, Anjanaharibe-Sud, Tsararano and Ambolokopatrika (Raxworthy et al. 1998; Andreone et al. 2000; Raselimanana et al. 2000) may refer to different species. Indeed, unpublished data collected by F. Andreone indicate that species occurring in this area are genetically different from all other *B. mandraka*-like forms, including *B. sambirano*. Our own observations at Ranomafana in south-eastern Madagascar indicate the presence, in that area, of a further bioacoustically distinct form. From a single species, *B. mandraka*, in 1979 (Blommers-Schlösser 1979b; Blommers-Schlösser & Blanc 1991), the number of species in this group of frogs has increased to four described and may include another two to three undescribed species.

The *B. mandraka* group, according to the data available at present, ranges in Madagascar from the south-eastern rainforests around Ranomafana (pers. obs. in 2004) to Marojejy in the North-East (Raselimanana et al. 2000, pers. obs. in 2005) and Manongarivo in the North-West (*B. sambirano*; this paper). No species in this group have so far been found in the northernmost rainforest of Montagne d’Ambre (Raxworthy & Nussbaum 1994; pers. obs.), nor in the Andohahela reserve in the extreme south (Nussbaum et al. 1999; pers. obs.).

Several of the species in the *B. mandraka* group appear to be restricted to particular biogeographic regions. *B. sambirano* has so far only been found in the North-West of Madagascar. It occurs in at least one reserve (Manongarivo Special Reserve) but may also be present in Tsaratana Strict Nature Reserve. Although these reserves harbour large forest blocks where deforestation is not yet an imminent problem, the status of *B. sambirano* should be carefully evaluated because its apparently restricted range may qualify this species for the category of “Near Threatened” according to IUCN threat criteria (IUCN 2001).

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