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A New Species of *Scaphiophryne* from Western Madagascar

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We describe the adult and larval morphology, advertisement call, ecology, and life history of a new species of Marbled Toad from the dry deciduous forests of western Madagascar on the basis of eight specimens from Kirindy Forest C. F. P. F. in the central Menabe area. *Scaphiophryne menabensis* n. sp. is larger, but morphologically similar to *S. marmorata* from the eastern rainforests. However, DNA sequence analysis of the mitochondrial 16S rRNA gene resulted in a clear differentiation from this species. The strongest mitochondrial affinities are with *S. madagascariensis*, a morphologically highly divergent species occurring in montane savanna and forest areas on the high plateau of Madagascar.

MARBLED Toads, genus *Scaphiophryne*, are medium-sized, mainly terrestrial anurans endemic to Madagascar. Together with the monotypic *Paradoxophyla* they have been classified in their own family Scaphiophrynidae (Dubois, 1992), but recent molecular work (van der Meijden et al., 2004) corroborated their inclusion in the Microhylidae as subfamily Scaphiophryninae (Blommers-Schlösser and Blanc, 1991). Most *Scaphiophryne* species are explosive breeders, often using shallow, temporary waters as breeding sites (Glaw and Vences, 1994).

Recently, the number of *Scaphiophryne* species was raised to seven including two previously unrecognized species (Vences et al., 2003). In contrast to the mantellid and cophyline radiations, which clearly show their maximum diversity in the humid forests of eastern Madagascar, *Scaphiophryne* appears to have diversified in the eastern, central, and western biogeographic regions of the island.

Within the genus, *Scaphiophryne marmorata* is unique as it is currently considered to occur in eastern rainforests as well as in remnants of western dry forests (Kirindy, Bemaraha, Namoroka, Isalo region; Vences et al., 2003). This is surprising, as these two types of forest are very different in respect to climate and seasonality, forest structure, and breeding site characteristics. Along the same line, recent work on other amphibians (Vences and Glaw, 2002) and reptiles (Nussbaum and Raxworthy, 1998; Nussbaum et al., 1998) has shown that eastern and western populations of several forms are distinct taxa. These examples of sister species distributed allopatrically in eastern and western Madagascar might be a result of vicariant speciation.

In agreement with these findings, *Scaphiophryne marmorata* from the west often differ from those of the east in their coloration. Specimens from the west are mostly brown with dark markings in contrast to those from the east which are green with dark markings. In a revision of the *Scaphiophryne marmorata* complex, however, Vences et al. (2003) stated that specimens from Kirindy Forest and from the Tsingy de Bemaraha in the west could not be reliably distinguished morphologically from eastern *S. marmorata.* However, in that study only a limited number of specimens were available for comparison, and it was not possible to assess the degree of genetic differentiation between eastern and western populations.

To clarify this unresolved issue, we tested for differentiation among eastern and western populations of *S. marmorata.* We here report on morphological differences between specimens of the two populations and provide a mitochondrial DNA sequence analysis. The surprising result was that the western species is genetically not related to eastern *S. marmorata* but rather to *S. madagascariensis*, a morphologically highly divergent species. We consider the observed differences as indicative of separation on the species level, and describe the morphology, advertisement call, ecology, and life history of *Scaphiophryne menabensis* n. sp.

MATERIALS AND METHODS

Three amplectant pairs and one single calling male were collected on 5 February 2002 between 0100–0300 h in the Kirindy Forest. These specimens were found floating on the water surface together with about 20 more individuals in a forest pond in the area locally known as CS5 after a heavy rainfall. Additionally, one calling male was collected on 13 January 2001 in a forest pond (CS7).

Photographs of representative frogs were taken soon after capture to record natural coloration (Fig. 1). The specimens were euthanized with MS222 and fixed and preserved in 70%

ethanol. Institutional abbreviations follow Leviton et al. (1985).

Body measurements were taken with calipers to the nearest 0.1 mm: SVL (snout-vent length), HW (maximum head width), HL (head length, from the maxillary commissure to the snout tip), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (distance between nostrils), HAL (hand length, from the carpal-metacarpal articulations to the tip of the longest, third, finger), FORL (forelimb length, from the axil to the tip of the longest finger), HIL (hindlimb length, from the cloaca to the tip of the longest, fourth, toe), FOL (foot length, from the tarsal-metatarsal articulations to the tip of the longest toe), FOTL (foot length including tarsus, from the tibiotarsal articulation to the tip of the longest toe), IMTL and IMTH (maximum length and height of inner metatarsal tubercle), FD4 (maximum width of terminal disk of fourth finger), RHL (relative hindlimb length, point reached by tibiotarsal articulation when hindlimb is pressed along the body), coded as follows: 0 = the tibiotarsal articulation does not reach the forelimb insertion; 1 = it reaches forelimb insertion; 2 = it reaches between forelimb and tympanic region; 3 = it reaches tympanic region. These measurements were compared to those taken on *S. marmorata* specimens from the east by Vences et al. (2003).

All specimens were assumed to be adults as they were observed to be engaged in breeding activities (amplexus or calling). Calls were recorded using a Sony WM TCD–100–S04 DAT–Recorder and Sennheiser microphone. Call parameters were analysed using Raven 1.0 software package (© Cornell Lab of Ornithology).

Muscle tissue samples were taken from freshly euthanized specimens in the field and preserved in pure ethanol. DNA was extracted using standard protocols and a fragment of the mitochondrial 16S rRNA gene amplified using the primers 16Sa-L and 16Sb-H (S. R. Palumbi, A. Martin, S. Romano, W. McMillan, L. Stice, and G. Grabowski, 1991, The Simple Fool's Guide to PCR, ver. 2, unpubl.), and protocols described by Vences et al. (2002b).

Phylogenetic analysis was carried out using PAUP* (vers. 4.0b10, D. L. Swofford, PAUP*: phylogenetic analysis using parsimony [*and other methods], Sinauer, Sunderland, MA, 2002). We performed unweighted maximum parsimony heuristic searches, with tree-bisection reconnection branch swapping, and random sequence addition with 100 replicates. In addition, a maximum likelihood analysis was performed after determining the substitution model that best fits our data through hierarchical likelihood ratio tests as implemented in Modeltest (Posada and Crandall, 1998). Robustness of nodes was tested by full heuristic bootstrapping, with 2000 pseudoreplicates (and 10 random addition sequence replicates) under maximum parsimony and 500 pseudoreplicates under maximum likelihood. A species of *Microhyla* (Microhylinae), and species of the genus *Paradoxophyla* that following Blommers-Schlösser and Blanc (1991) is the closest relative of *Scaphiophryne*, were used as outgroups.

Clutch size was determined by counting the eggs of one amplectant pair that we collected in the field and kept over night in an aquarium where it spawned. We measured ovum diameter in a sample of 20 eggs from each clutch of two distinct clutches.

Tadpoles of *Scaphiophryne* were collected in the field from January to March 2001 and 2002 in the Kirindy Forest. Additionally, fertilized eggs from one amplectant pair were reared in plastic aquaria filled with rainwater. Tadpoles were fed ad libitum with commercial fish food (TetraMinTabs®). We preserved 12 tadpoles in different developmental stages in 5% formalin (ZSM 358/2004). Staging is according to Gosner (1960) and nomenclature of morphological features follows McDiarmid and Altig (1999). Measurements of morphometric variables were taken from preserved specimens using a stereo microscope (Zeiss® Stemi SV 6) with a measuring ocular. Drawings were done with a camera lucida. Froglet size at metamorphosis was recorded on specimens that were caught in late developmental stages (≥ Gosner 40) in the field and raised to complete metamorphosis (Stage 45–46; n = 10, ZSM 628/2003). These specimens were euthanized with chlorobutanol subsequent to measurements and preserved in 5% formalin. Measurements were taken to the nearest 0.05 mm with calipers.

Water chemistry parameters of tadpole habitats were measured using WA 300 conductivity analyser (Conrad Electronics), DO–5509 Oxygen analyser (Conrad Electronics) and pHep® pH analyser (Hanna Instruments). Sympatric tadpole species were identified in the natural ponds and breeding habitat choice was assessed by repeatedly sampling 200 natural ponds with the box-method and by standardized dip-netting (Heyer et al., 1994).

Scaphiophryne menabensis, new species

Figure 1, Table 1

Holotype.—ZSM 186/2003, adult male, western Madagascar, province of Toliara, district of Mo-

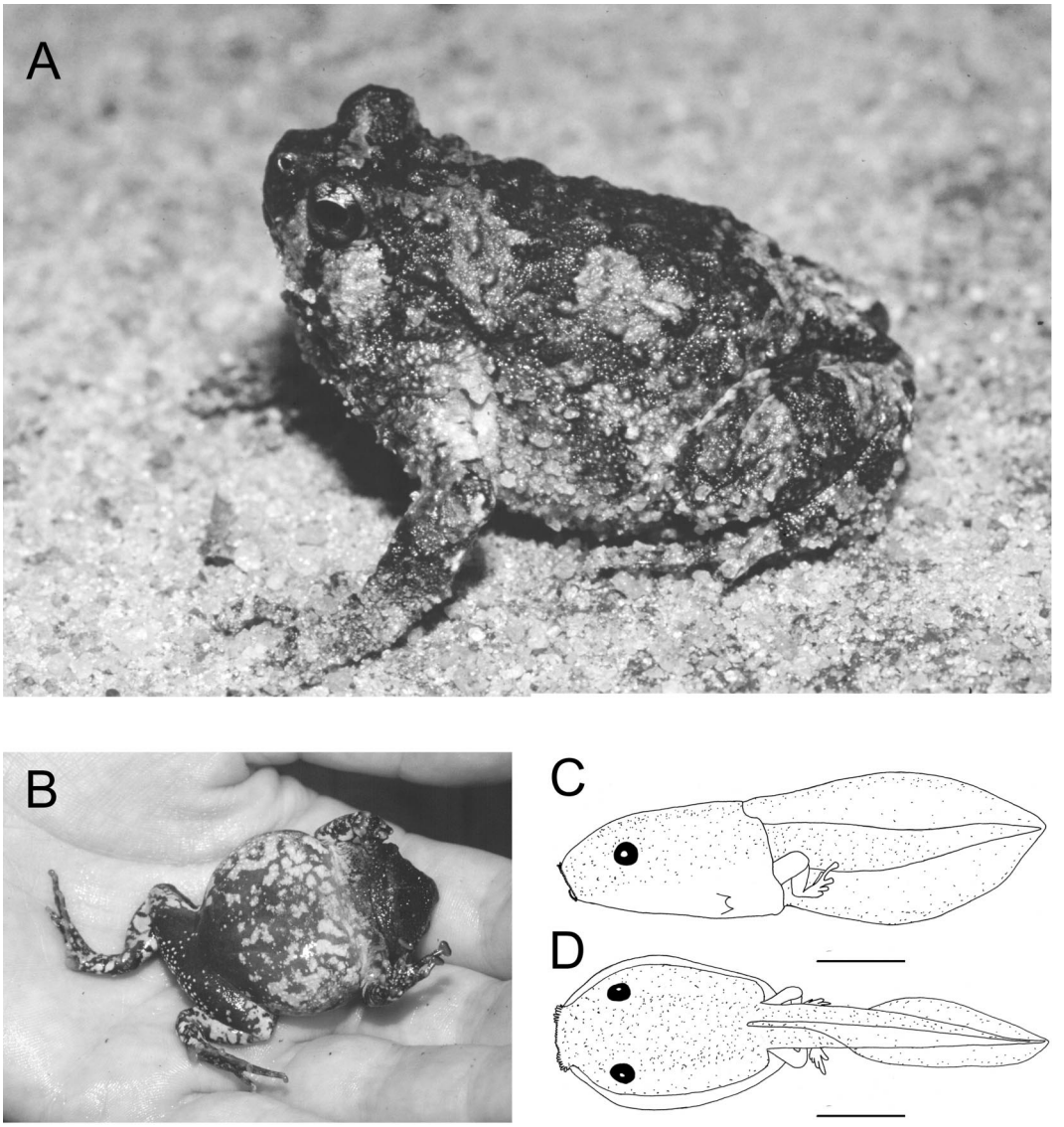

Fig. 1. (A) Dorsolateral and (B) Ventral views of living adult specimen of *Scaphiophryne menabensis* n. sp. For color photograph see Glos (2003). (C) Lateral view and (D) Dorsal view of a *Scaphiophryne menabensis* n. sp. tadpole at stage 39. Scale bars represent 5 mm.

rondava, in the Kirindy Forest C. F. P. F. (forest pond CS5), 44°39′E, 20°03′S, 18–40 m elevation, J. Glos, 5 February 2002.

Paratypes.—ZSM 187/2003, ZSM 188/2003 and ZSM193/2003, three adult females, and ZSM 189/2003, ZSM 190/2003, and ZSM 192/2003, three adult males, western Madagascar, province of Toliara, district of Morondava, in the Kirindy Forest C. F. P. F. (forest pond CS5), J. Glos, 5 February 2002. ZSM 191/2003, one adult male, Kirindy Forest C. F. P. F. (forest pond CS7), J. Glos, 13 January 2001. UMMZ 219487, one adult male, Kirindy Forest, C. J. Raxworthy, J. B. Ramanamanjato, A. Raselimanana, A. Razafimanantsoa, and A. Razafimanantsoa, 31 January 1996, specimen examined by Vences et al. (2003).

Diagnosis.—A medium sized *Scaphiophryne*; SVL of adult males 40.3–42.0 mm (n = 5), of adult females 42.6–45.2 mm (n = 3). *Scaphiophryne menabensis* differs from *S. brevis*, *S. calcarata*, and *S. madagascariensis* by highly expanded terminal disks on the fingers and toes (vs. not or only slightly enlarged); from *S. gottlebei* by a very dif-

TABLE 1. MORPHOMETRIC MEASUREMENTS (MM) OF ADULT SPECIMENS OF *Scaphiophryne menabensis* N. SP. M = MALE, F = FEMALE. For abbreviations of measured variables see Materials and Methods section.

Specimen	Sex	SVL	HW	HL	ED	END	NSD	NND	HAL	FORL	HIL	FOL	FOTL	IMTL	IMTH	FD4	RHL
ZSM 186/2003	M	40.5	12.7	11.0	3.5	3.1	1.5	3.0	13.1	27.9	52.1	19.4	25.2	4.1	2.7	2.6	3
ZSM 187/2003	F	45.2	13.7	12.9	3.6	3.4	1.9	3.0	13.7	30.0	55.4	19.5	26.7	3.8	2.8	2.7	2
ZSM 188/2003	F	43.1	13.2	11.4	3.4	2.9	1.9	3.2	13.2	30.8	53.3	19.6	25.5	4.1	2.8	2.3	3
ZSM 189/2003	M	40.4	12.4	10.8	4.2	3.3	1.4	2.4	12.4	27.3	50.1	18.4	25.2	3.0	2.3	2.1	2
ZSM 190/2003	M	40.3	11.9	11.9	3.0	3.4	1.9	2.6	13.0	29.4	51.1	18.8	25.6	4.0	2.8	2.3	3
ZSM 191/2003	M	42.0	12.5	11.2	3.0	3.0	1.9	2.8	12.1	28.1	49.1	18.3	23.3	4.1	2.4	2.2	2
ZSM 192/2003	M	41.3	13.2	11.9	3.8	3.5	2.5	3.4	11.9	29.6	51.8	19.4	26.4	4.0	2.5	2.4	2
ZSM 193/2003	F	42.6	14.5	11.7	3.6	3.2	1.8	3.1	14.0	29.8	53.8	20.8	27.2	4.3	2.1	2.6	3

ferent coloration (without distinct black, red, green, and white areas as visible in living and ethanol-preserved *S. gottlebei*) and granular dorsal skin (vs. smooth); from *S. boribory* by smaller snout-vent length (47–60 mm vs. 40–45 mm), granular dorsal skin, and coloration; from *S. spinosa* by coloration and by the absence of large dermal spines above forelimbs, at maxilla commissure, and in the tympanic region. *Scaphiophryne menabensis* most resembles *S. marmorata*, but differs by a larger snout-vent length (males of *S. marmorata* 32.4–35.9 mm, females 34.9–43.5 mm), a narrower head, shorter relative hindlimb length, shorter relative hand length, a longer inner metatarsal tubercle, and a brownish dorsal coloration (Tables 1, 2).

Scaphiophryne menabensis furthermore differs from all other *Scaphiophryne* species except *S. madagascariensis* by a significant genetic differentiation in the 16S mitochondrial gene and probably from *S. spinosa*, *S. boribory*, and *S. madagascariensis* by shorter advertisement calls.

Description.—Adult male in breeding state; specimen in excellent state of preservation. For morphometric measurements see Table 1. Medium sized, stout frog; snout rounded in dorsal and lateral profile, nostrils directed laterally, closer to snout than to eye; horizontal pupil; tympanum not visible; single, subgular vocal sac; tongue ovoid; maxillary teeth and vomerine teeth absent. Arms slender; fingers without webbing; relative length of fingers $1 < 2 = 4 < 3$; small subdigital tubercles; no metacarpal tubercles. First toe short, fourth toe much longer than third and fifth toe, third toe as long as fifth toe; relatively large inner metatarsal tubercles; minute webbing between toes. Tibiotarsal articulation reaches tympanic region. Skin on dorsum with two rows of larger tubercles and many small granules, without dorsolateral folds; absence of large tubercles above forelimb insertion and in tympanic region; prominent tubercle before hindlimb insertion. Ventral skin slightly granular; legs dorsally very slightly granular, ventrally not granular.

Dorsal coloration in life is brown with symmetrical darker markings, including feet and arms (Fig. 1A). Ventral pattern with contrasted dark brown–light cream marbling, extending on legs and arms (Fig. 1B); in the region of inner thighs smaller pattern of marbling with a higher proportion of brown. Color of throat is dark brown, with many small granules.

Variation.—ZSM 187–193/2003 were examined (Table 1) and directly compared with the holotype. All frogs are largely concordant in mor-

TABLE 2. RELATIVE BODY PROPORTIONS (MEAN ± SD) OF *Scaphiophryne menabensis* N. SP. AND *S. marmorata*. For abbreviations see Materials and Methods section. Data for *S. marmorata* are from Vences et al. (2003). Bold indicates significant differences between *S. menabensis* and *S. marmorata* ($\alpha = 0.05$). Data for males and females were analyzed separately; Mann-Whitney U-Test.

	Males				Females			
	S. menabensis N = 5	*S. marmorata* N = 6	Z	P	*S. menabensis* N = 3	*S. marmorata* N = 6	Z	P
SVL (mm)	**40.9 ± 0.7**	**34.8 ± 1.5**	**−2.74**	**<0.01**	43.6 ± 1.1	38.8 ± 2.8	−1.81	0.09
HW/SVL	**0.31 ± 0.01**	**0.35 ± 0.02**	**−2.74**	**<0.01**	0.32 ± 0.02	0.34 ± 0.02	−1.29	0.26
HL/HW	**0.91 ± 0.05**	**0.82 ± 0.04**	**−2.19**	**0.03**	0.87 ± 0.06	0.84 ± 0.02	−0.77	0.55
ED/SVL	**$8.59 \pm 1.24 \times 10^{-2}$**	**$10.34 \pm 0.62 \times 10^{-2}$**	**−2.19**	**0.03**	$8.13 \pm 0.30 \times 10^{-2}$	$8.73 \pm 0.99 \times 10^{-2}$	−0.52	0.71
NSD/SVL	$4.44 \pm 0.88 \times 10^{-2}$	$3.93 \pm 0.39 \times 10^{-2}$	−0.73	0.54	**$4.32 \pm 0.09 \times 10^{-2}$**	**$3.41 \pm 0.43 \times 10^{-2}$**	**−2.32**	**0.02**
NND/SVL	6.90 ± 0.78	7.52 ± 0.47	−1.28	0.25	7.09 ± 0.41	7.64 ± 0.82	−0.52	0.71
HAL/SVL	**0.31 ± 0.02**	**0.35 ± 0.01**	**−2.74**	**<0.01**	0.31 ± 0.01	0.34 ± 0.02	−1.81	0.09
FORL/SVL	0.70 ± 0.02	0.72 ± 0.02	−0.91	0.43	0.69 ± 0.02	0.69 ± 0.03	−0.26	0.90
HIL/SVL	**1.24 ± 0.04**	**1.36 ± 0.04**	**−2.74**	**<0.01**	**1.24 ± 0.02**	**1.37 ± 0.07**	**−2.32**	**0.02**
IMTL/SVL	$9.37 \pm 0.95 \times 10^{-2}$	$8.76 \pm 0.88 \times 10^{-2}$	−1.46	0.18	$9.31 \pm 0.73 \times 10^{-2}$	$8.29 \pm 0.61 \times 10^{-2}$	−1.81	0.09
FD4/HAL	$5.66 \pm 0.38 \times 10^{-2}$	$5.65 \pm 0.37 \times 10^{-2}$	−2.74	1.00	$5.81 \pm 0.73 \times 10^{-2}$	$5.53 \pm 0.61 \times 10^{-2}$	−1.29	0.26

phology and coloration, however, the female specimens (ZSM 187, 188, 192/2003) are larger than the males. In life some specimens had small green markings behind the forelimb insertion.

Distribution.—The type locality is the Kirindy Forest C. F. P. F. (Centre de Formation Professionelle Forestière), a deciduous dry forest near the west coast of Madagascar, 60 km northeast of Morondava and about 20 km inland (44°39′E, 20°03′S; 18–40 m elevation; Sorg and Rohner, 1996). The area of the Kirindy forest covers about 12,000 ha and thus may be among the largest remaining continuous forests in western Madagascar (Nelson and Horning, 1993). In this forest, the new species was found in the areas locally known as CS5, CS6, and CS7. Surveys in the Menabe region in January and February 2004 at five sites west of the Kirindy Forest and between the Kirindy Forest and Bemaraha (J. Glos, unpubl. data) and in a distance of 5 to 30 km from Kirindy were unsuccessful in finding this species. We attribute three other localities to this species: (1) the ''Tsingy de Bemaraha'' Reserve (18°42.481′S, 44°42.981′E), UMMZ 219488–219489 and 219491–219495, 8–11 March 1996, C. J. Raxworthy, J. B. Ramanamanjato, A. Raselimanana, A. Razafimanantsoa, and A. Razafimanantsoa; (2) the Namoroka Reserve about 200 km further north (16°28.189′S, 45°20.906′E), UMMZ 227499, 6 December 1996, C. J. Raxworthy, J. B. Ramanamanjato, A. Raselimanana, A. Razafimanantsoa, and A. Razafimanantsoa; and (3) the Isalo region, UMMZ 227489, local collector. Specimens from these localities agreed morphologically with those from Kirindy. However, since no genetic data are available for the populations from Bemaraha, Namoroka, and Isalo, and only single specimens were collected from the latter two localities, their attribution to *S. menabensis* requires further confirmation.

Molecular phylogenetic relationships.—As stressed by Vences et al. (2002b, 2003), the mitochondrial differentiation among species of *Scaphiophryne* is surprisingly low. Of a total of 528 characters included in the analysis, 413 were constant and only 77 were parsimony-informative. Figure 2 shows a strict consensus of the four most parsimonious trees found; length of these was 217 steps, consistency index was 0.714 and retention index was 0.791. Most clades received very low bootstrap support, probably caused by the limited number of informative sites. *Scaphiophryne menabensis* was placed with moderate bootstrap support (74% in the maximum par-

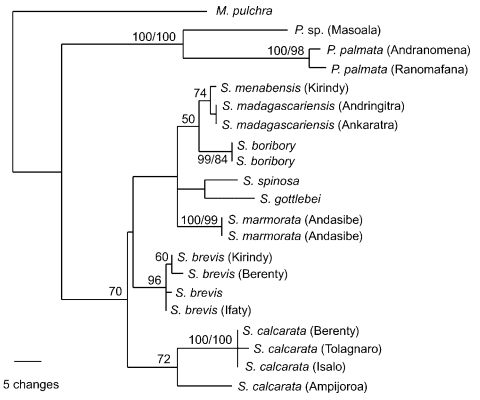

Fig. 2. Strict consensus phylogram of four most parsimonious trees obtained by maximum parsimony searches based on 528 nucleotides of the mitochondrial 16S rRNA gene. First numbers are bootstrap support values from a maximum parsimony bootstrap analysis (2000 replicates). Second numbers are bootstrap support values in percent from a maximum likelihood search, based on a RTrN + I + G nucleotide substitution model selected by Modeltest, with empirical base frequencies and sustitution rates, a proportion of invariable sites of 0.5662, and a gamma distribution shape parameter of 0.4066. Where only single numbers are given, they refer to the parsimony bootstrap analysis. Values below 50% are not shown. Collecting localities are shown for specimens with reliable data; others were obtained from commercial collectors. Abbreviations: *S.* = *Scaphiophryne*, *P.* = *Paradoxophyla*, *M.* = *Microhyla*.

simony analysis) as sister clade of *S. madagascariensis*. Two individuals of the latter species, from the Ankaratra and Andringitra Massifs, respectively, had fully identical sequences despite their different morphology reported by Vences et al. (2002a). The differentiation of *S. menabensis* to *S. madagascariensis* was only 0.4% pairwise sequence divergence (two different nucleotides over the whole gene fragment), whereas the difference of *S. menabensis* to *S. marmorata* was of 2.9% (15 nucleotide differences).

Natural history.—*Scaphiophryne menabensis* is an explosive breeder that breeds only after heavy rainfalls (> 40 mm), and only a few times during the rainy season (December to March; data from the rainy seasons 2000/2001, 2001/2002, 2002/2003, 2004). Males call while floating on the water exclusively at night. Several pairs were observed in axillary amplexus. Within the Kirindy forest, *S. menabensis* is among the rarest anuran species. Of more than 200 potential breeding waters that we investigated in the Kirindy Forest (J. Glos, unpubl. data), calling *S. menabensis* and/or its tadpoles were found only at three different pools, all of them medium sized (range 123–235 m^2), ephemeral waters in the closed forest. *Scaphiophryne menabensis* has a small spatial niche; it was neither found in rock pools of the river bed (before the river is running), nor in savanna ponds, although these types of breeding waters are abundant around the Kirindy Forest. All breeding ponds had similar characteristics. They were shallow with a water depth of less than 10 cm over 50–95% (range, n = 3) of their surface. Maximum water depth was 21–45 cm when the ponds were completely water-filled. Water was clear to slightly muddy. There was very low coverage of aquatic vegetation (floating water plants, grasses, underwater vegetation). Water chemistry was comparable in these three ponds (oxygen concentration: 1.28–1.42 mg/l, conductivity 100–161 μS, pH 6.55–6.66). Compared to the other 200 breeding waters known in Kirindy, invertebrate predator load was small.

The following syntopic tadpole species were found: *Boophis doulioti*, *B. xerophilus*, *Aglyptodactylus laticeps*, *Mantella betsileo*, *Dyscophus insularis*, *Scaphiophryne calcarata*.

We never found adult *S. menabensis* during the day. Captured specimens were observed to be fairly good climbers.

Life history.—Eggs were deposited as a single layered surface film. Clutch size in one female was 450 eggs, ovum diameter was 2.21 ± 0.21 mm (mean ± SD; range 1.68–2.72 mm; n = 40 from two amplectant pairs that spawned in the field camp). One dissected female (ZSM 193/2003) contained 670 oocytes (diameter 1.51 ± 0.07; range 1.41–1.63). Snout-vent length at metamorphosis was 10.3 ± 0.7 mm (range 9.4–11.4 mm, n = 10).

Advertisement call.—The call consists of a series of short notes (Fig. 3). At 25 C, call duration was 0.51–0.76 sec (n = 3). Calls are repeated with an interval of 0.61–0.77 sec. Calls contained 13–19 notes (n = 5). Note duration was 16–17 ms (n = 6). Intervals between notes were 17–24 ms (n = 6). The intensity of notes was constant throughout the call. Frequency was 500–1400 Hz; the dominant frequency was 850 Hz. The advertisement call of *S. menabensis* is structurally similar to that of *S. spinosa*, *S. boribory*, and *S. madagascariensis* as described by Vences et al. (2002a, 2003). However, call and single notes appear to be shorter in *S. menabensis* as compared to the other species. In *S. madagascariensis*, note duration was 28–32 ms (Vences et al., 2002a), in *S. spinosa*, call duration was 3539–9117 ms and note duration was 25–34

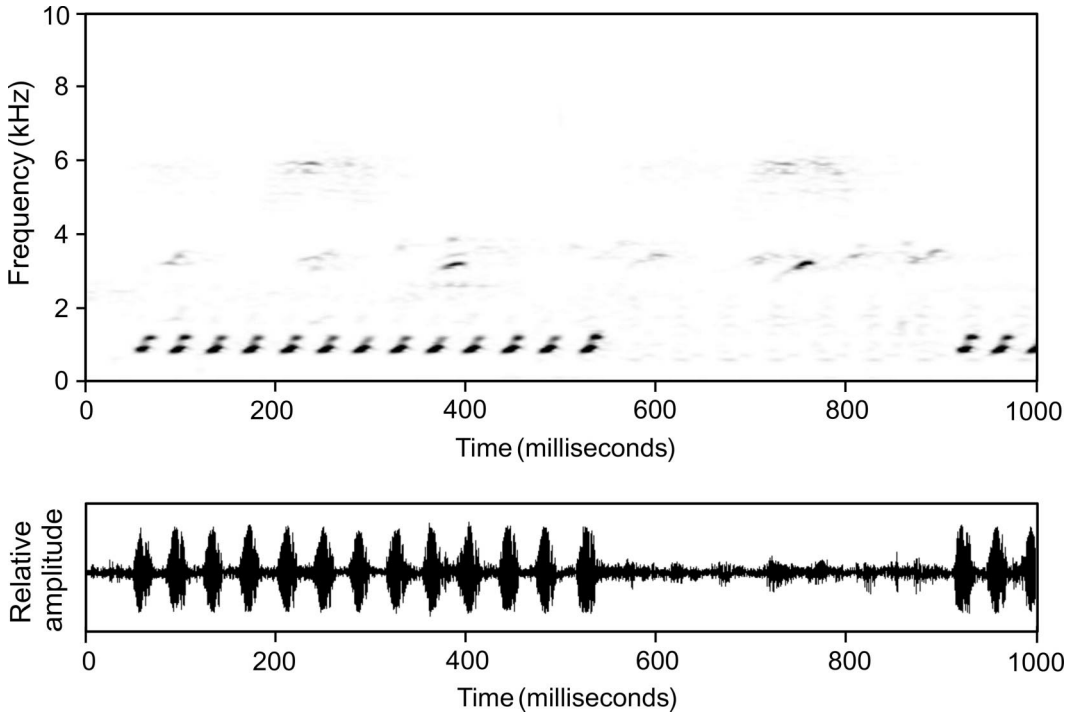

Fig. 3. The advertisement call of *Scaphiophryne menabensis* n. sp. Audiospectrogram (sonogram: frequency in kHz vs. time) and oscillogram (relative amplitude vs. time).

ms, and in *S. boribory*, call duration was up to 12,492 ms and note duration 35–38 ms (Vences et al., 2003). Although these variables may be influenced by temperature (recorded at a temperature range as wide as from 16 C in *S. madagascariensis* to 25 C in *S. spinosa*), this is unlikely to be the only explanation for the differences of *S. menabensis* to the other species. Because the calls of *S. marmorata* are unknown, the existing bioacoustic data cannot be used to assess possible differences between *S. menabensis* and this species. Among sympatric microhylids at Kirindy, the call of *Scaphiophryne menabensis* is very different compared to those of *S. brevis* and *S. calcarata* but can be mistaken with that of *Dyscophus insularis*.

Description of tadpole.—Because the tadpoles showed no considerable ontogenetic changes in oral disc structure and body proportions (see Table 3), we give data as mean and standard deviation for tadpoles of all developmental stages pooled together.

Body of the tadpoles depressed ovoid in lateral and wide ovoid in dorsal view (Fig. 1C, D). Laterally, there is a considerable gap between the outer integument and the body with no visible tissue, giving the tadpole a broad, disc-like appearance. Snout flat in dorsal and lateral profile, eyes medium sized and directed dorsolaterally. Nostrils dorsal, in the same distance to snout tip and to eyes (ratio naris-eye distance/snout-naris distance 1.02 ± 0.21, mean ± SD), spiracle sinistral, at the rear of the body, low, inner lateral wall absent, directed posteriorly. Medial vent tube short, aperture dextral.

Tail sturdy; tail fins convex, higher from the posterior end of vent tube to the middle of the tail, dorsal fin as high as ventral fin. Tail tip rounded. Origin of dorsal fin slightly before the base of tail.

Oral disc directed anteriorly, no tooth rows. Marginal papillae conical, with rounded tips, slightly pigmented; in stage 39 length of marginal papillae 0.18 mm, density 7.6 papillae/mm. Upper jaw sheath concave, lower jaw sheath V-shaped, both sheaths not serrated and not pigmented.

In vivo, dorsal color is lightly brownish, ventral color is white. Caudal musculature is entirely pigmented, dorsal tail fin heavily pigmented dorsally and only slightly pigmented ventrally, ventral tail fin only slightly pigmented dorsally and heavily pigmented ventrally.

Etymology.—The specific name refers to the Menabe region in which the type locality Kirindy Forest is situated (Malagasy for Menabe =

TABLE 3. MEASUREMENTS (MM) OF TADPOLES OF *Scaphiophryne menabensis* N. SP. Staging after Gosner (1960), measurements follow McDiarmid and Altig (1999). BH = body height, BL = body length, BW = body width, ED = eye diameter, IND = internarial distance, IOD = interorbital distance, MTH = maximum tail height, NED = naris–eye distance, ODW = oral disc width, SED = snout–eye distance, SND = snout–naris distance, SSD = snout–spiracle distance, TL = total length, TMH = tail musculature height, TMHM = tail musculature height at midlength of tail, TMW = tail muscle width.

	Mean ± SD	Range
Stage 25–28 (N = 7)		
TL	15.21 ± 2.77	10.15–18.36
BL	6.08 ± 1.05	4.00–7.24
ED	0.54 ± 0.16	0.29–0.83
SSD	5.15 ± 0.97	3.30–6.48
ODW	1.62 ± 0.34	0.97–2.04
Stage 36–39 (N = 5)		
TL	27.00 ± 2.67	22.90–31.00
BL	11.87 ± 0.79	11.00–12.96
ED	0.92 ± 0.15	0.71–1.11
SSD	9.96 ± 0.22	9.72–10.26
ODW	3.34 ± 0.25	3.08–3.80
All stages pooled (N = 12)		
BL/TL	0.42 ± 0.03	0.38–0.48
BL/BW	1.29 ± 0.18	1.14–1.84
BL/BH	1.72 ± 0.22	1.41–2.02
BW/BH	1.35 ± 0.22	0.83–1.64
TMHM/MTH	0.28 ± 0.04	0.21–0.35
IOD/IND	3.48 ± 0.81	2.18–4.71
SND/SED	0.59 ± 0.07	0.43–0.64
Dorsal/Ventral fin	0.94 ± 0.12	0.79–1.20
NED/BL	0.21 ± 0.02	0.17–0.25
TMH/BH	0.31 ± 0.04	0.24–0.38
TMW/BW	0.22 ± 0.05	0.14–0.35

"big red"). This name was chosen to highlight the unique status of the Menabe region as local center of biodiversity and as one of the largest remnants of the Malagasy dry forest.

DISCUSSION

Relationships.—Among *Scaphiophryne*, and based on morphological features, *S. menabensis* clearly shows the strongest affinities to *S. marmorata*. These two species share their moderately granular skin, enlarged disks of fingers and toes, and size. Although they have different color patterns, with a basically brown color in *S. menabensis* and a mainly green pattern in *S. marmorata*, the chromatic differences among them are smaller than those distinguishing other species (e.g., the conspicuous *S. gottlebei*). Nevertheless, the analysis of 16S rDNA sequences leaves little doubt that the mitochondrial (maternal) affinities of *S. menabensis* are with *S. madagascariensis*, a species typically occurring in montane savanna and forest areas on the high plateau of Madagascar. Although the genetic divergence among *S. madagascariensis* and *S. menabensis* is extremely low, these two species are morphologically very distinct. They also appear to slightly differ in advertisement calls, which is relevant because *Scaphiophryne* appear to be characterized by a highly conserved evolution of call features (Vences et al., 2003).

Vences et al. (2002b) demonstrated that *Scaphiophryne gottlebei*, the second *Scaphiophryne* with enlarged finger disks occurring in western Madagascar, is an allotetraploid species that originated by hybridization. Hybridization is likely in explosively breeding species with similar advertisement calls, such as *Scaphiophryne*, and phenomena of recent mitochondrial introgression could possibly account for the fact that *S. menabensis* is mitochondrially very closely related to *S. madagascariensis* whereas morphologically it seems closer to other taxa. Analysis of nuclear markers and karyotypes will be necessary to clarify this enigma, and any phylogeographic discussion on the origin of *S. menabensis* will have to await these data.

Life history.—*Scaphiophryne menabensis* can be considered a typical explosive breeding species reproducing only one or a few times per season in lentic, temporary waters. This is also true for most *Scaphiophryne* species. In contrast to other *Scaphiophryne* and in particular to *S. marmorata* that lay a large number of small eggs (*S. marmorata* 1.2 mm egg diameter, *S. boribory* 1.7 mm, *S. spinosa* 1.4–1.7 mm; Blommers-Schlösser, 1975; Vences et al., 2002a, 2003), *S. menabensis* lays fewer and relatively large eggs. Large clutches and small egg sizes are regularly found throughout seasonal areas of the tropics, and are seen as an r-strategy in this often unpredictable environment. However, *S. menabensis* clutches with a relatively low number of large eggs can be regarded as an alternative strategy to cope with rapidly dessicating breeding waters. A larger size of oocytes and probably also of hatchlings may give *S. menabensis* tadpoles a head start in larval growth and development, and may increase the chance to complete metamorphosis before the breeding site dries up.

In general, the tadpoles of *S. menabensis* are morphologically similar to tadpoles of other *Scaphiophryne* species (e.g., *S. brevis*, *S. calcarata*; Blommers-Schlösser, 1975; J. Glos pers. obs.). These tadpoles are unique in their morphology

as they are intermediate between the ranoid and the microhylid type (Wassersug, 1984). They are easily distinguishable in the field from other tadpoles as they have a very broad, almost disc-like appearance caused by a gap with no apparent tissue between the main body and the outer integument.

Distribution and conservation.—*Scaphiophryne marmorata* is mainly restricted to humid forests of the central east of Madagascar (Glaw and Vences, 1994). In contrast, current knowledge of geographic distribution patterns in the genus *Scaphiophryne* suggests that *S. menabensis* is restricted to the dry deciduous forests of the central west. The type locality, Kirindy C. F. P. F., as well a second site, the natural reserve of the ''Tsingy de Bemaraha,'' are privately and officially protected areas, respectively. These sites represent some of the largest and most pristine areas of the highly endangered and fragmented dry forest ecosystem of western Madagascar. Namoroka Reserve, north of Bemaraha, might be a third locality. The identity and precise location of a fourth population in the Isalo region in the south remains unknown, but the large size of a female specimen (SVL 48.5 mm; Vences et al., 2003) might justify a preliminary determination as *S. menabensis* rather than *S. marmorata*. In a herpetological survey in 2004 at five sites between the Kirindy area and Bemaraha and around Kirindy C. F. P. F., no *S. menabensis* were found (J. Glos, unpubl. data). The sites of this survey, however, are clearly more disturbed than the Kirindy Forest C. F. P. F., indicating that *S. menabensis* prefers relatively undisturbed dry forest habitat.

The dry forest of western Madagascar ranks among the world's most endangered ecosystems. In 1990, only 2.8% of its original area remained (Laurance and Bierregaard, 1997). Within this ecosystem, *S. menabensis* seems to be restricted to the relatively largest and least disturbed remnants of dry forest, Kirindy and Bemaraha. There are promising efforts to establish an integrated conservation concept for the Menabe region including the Kirindy Forest. Bemaraha is already under governmental protection and is comparatively well protected because of its remoteness. In addition to its apparently restricted distribution, *S. menabensis* was never abundant within its known sites of occurrence, and therefore might be a rare and threatened species.

Conclusion.—Up to now, *Scaphiophryne marmorata* was considered to be distributed both in the humid east and the arid west of Madagascar, as was the case for other anuran and reptile species. However, the prevailing climatic conditions in these two areas are very different. The central mountain chain, spanning from the north to the south of Madagascar, most likely acts as a dispersal barrier for frogs with low altitude distribution and inhibits gene flow between the east and the west. Even if climatic conditions in the past might have been similar in the two areas, the isolation of western populations following climatic shifts may have led to vicariant speciation. Along these lines, recent work on amphibians and reptiles detected several cases where vicariant sister taxa occur in eastern and western Madagascar (Nussbaum et al., 1998; Nussbaum and Raxworthy, 1998; Vences and Glaw, 2002), and a similar hypothesis could explain the origin of *S. marmorata* and *S. menabensis*. However, the results obtained so far reject this simple hypothesis, at least from a mitochondrial perspective. Speciation in *Scaphiophryne* might have been characterized by complex patterns, including recurrent hybridization, introgression, and admixture among forms occurring in the arid west, montane savannas, and eastern rainforests.

Material Examined

Genbank accession numbers of the sequences used are as follows: *Scaphiophryne boribory*: AJ314810 and AY594126; *S. brevis*: Kirindy, AF215384 and AY834194; Berenty, AY834195; Ifaty, AJ314808; *S. calcarata*: Ampijoroa, AJ314811; Berenty, AY834192; Tolagnaro, AY834193; Isalo region, AY594127; *S. gottlebei*: AF215385; *S. madagascariensis*: Ankaratra, AJ314809; Andringitra, AY834190; *S. marmorata*: Andasibe, AJ417567 and AY834191; *S. menabensis*: Kirindy, AY834189; *S. spinosa*: AF215383. *Microhyla pulchra*, Vietnam, AF215374; *Paradoxophyla palmata*: Andranomena, AY834187; Ranomafana, AY834188; *P.* sp.: Masoala, AY834186; *Dyscophus antongili*: Maroantsetra, AY834196; *D. guineti*: Fierenana, AY834197; *D. insularis*: Antsirasira, AY834198.

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