A new microhylid frog of the genus *Cophyla* from a transitional forest in northwestern Madagascar

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We describe a new species of the genus *Cophyla* from Berara forest, Sahamalaza Peninsula, northwestern Madagascar. This arboreal microhylid frog is morphologically similar to the single known species of the genus, *Cophyla phyllodactyla,* and to *Platypelis occultans*. This suggests that the latter species may in fact also belong to the genus *Cophyla,* a hypothesis that needs testing by osteological study. The new species is diagnosed by having an intermediate body size and longer notes in advertisement calls compared to these two species, its note duration being the longest recorded from any other cophyline species. In addition we provide evidence for a considerable genetic differentiation among these taxa, based on DNA sequences of the mitochondrial 16S rRNA gene. The new species of *Cophyla* is so far only known from primary transitional forest in northwestern Madagascar. It might be a regional endemic that is vulnerable to habitat destruction.

Key words: **Amphibia, Microhylidae, *Cophyla,* new species, Madagascar, phylogeny, taxonomy.**

INTRODUCTION

The microhylid frogs of Madagascar are classified in three subfamilies (Cophylinae, Dyscophinae, Scaphiophryninae), of which the Cophylinae is most species-rich. About 40 nominal species in seven genera are currently known in this subfamily. Cophylines are likely to represent a monophyletic group based on their derived reproductive behaviour (non-feeding tadpoles and parental care) and uniform advertisement call structure (long series of single melodious notes in almost all species) (Glaw & Vences 1994).

The genera *Madecassophryne* Guibé, 1974, *Plethodontohyla* Boulenger, 1882, *Rhombophryne* Boettger, 1880, and *Stumpffia* Boettger, 1881, contain mostly terrestrial or fossorial forms, whereas the remaining three cophyline genera are largely specialized to arboreal habits: *Anodonthyla* Müller, 1892 (with exception of the terrestrial *A. montana* Angel, 1925), *Platypelis* Boulenger, 1882 and *Cophyla* Boettger, 1880. While *Anodonthyla* are relatively well characterized by the presence of a distinct prepollex in males and the absence of vomerine teeth, the distinction between *Cophyla* and *Platypelis* is less clear and mainly based on the reduction of the vomer and osteological characters of *C. phyllodactyla* Boettger, 1880, the single *Cophyla* known to date (Blommers-Schlösser & Blanc 1991).

During fieldwork in the transitional forest of the Sahamalaza Peninsula in northwestern Madagascar we collected specimens of an arboreal microhylid that were superficially similar to *Cophyla phyllodactyla* but had much longer note durations in their advertisement calls (Andreone *et al.* 2001). Here we present molecular data that demonstrate that this form is indeed related to *C. phyllodactyla*. However, the large genetic, bioacoustic and morphological differentiation warrant a distinctness at the species level, and we therefore describe the Berara form as a new species.

MATERIALS & METHODS

Specimens were collected at night by localizing calling males. They were euthanatized by immersion in chlorobutanol, fixed in 5% formalin and preserved in 70% ethanol. Specimens are deposited in the Museo Regionale di Scienze Naturali, Torino (MRSN) and the Zoologische Staatssammlung München (ZSM).

Samples of muscle tissue were extracted from fresh specimens and preserved in 98% ethanol. DNA was extracted and a fragment of the mitochondrial 16S rRNA gene amplified and sequenced using standard protocols and primers (Vences *et al.* 2000). Sequences were analysed using PAUP*, version 4b10 (Swofford 2002), with heuristic searches under the maximum likelihood optimality criterion, after selecting a substitution model with

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Fig. 1. Holotype of *Cophyla berara* (ZSM 410/2000).

the program Modeltest (Posada & Crandall 1998).

DNA sequences were deposited in Genbank; voucher specimens and accession numbers are as follows: *Cophyla phyllodactyla* (Nosy Be; ZSM 460/2000; AY730867); *Cophyla* sp. n. (see below) (Berara; ZSM 410/2000; AY594093); *Platypelis barbouri* (Andasibe; ZSM 1/2002; AY594098); *Platypelis grandis* (Mantady; ZSM 162/2002; AY594102); *Platypelis milloti* (Manongarivo; ZSM 817/2003; AY594103); *Platypelis occultans* (Marojejy; MRSN A2660; AY594100); *Platypelis tuberifera* (Andasibe; ZSM 2/2002; AY730868); *Scaphiophryne calcarata* (Isalo; ZSM 118/2002; AY594127).

The following morphological measurements were taken by M.V. to the nearest 0.1 mm using a calliper: snout–vent length, SVL; maximum head width (HW); head length from tip of snout to posterior edge of snout opening (HL); horizontal tympanum diameter (TD); horizontal eye diameter (ED); distance between anterior edge of eye and nostril (END); distance between nostril and tip of snout (NSD); distance between both nostrils (NND); forelimb length, from limb insertion to tip of longest finger (FORL); hand length, to the tip of the longest finger (HAL); hindlimb length, from the cloaca to the tip of the longest toe (HIL); tibia length (TIL); foot length including tarsus (FOTL); foot length (FOL).

Calls were recorded using a Sony WM D6C professional walkman with external Vivanco microphone. They were digitized on a PC using the software Cooledit 96 (Syntrillium Corp.), with a sampling rate of 32 kHz. For visual representation of the sonagram, the following settings were used: Hanning Window (resolution 2048 bands) with linear energy plot (0.8% scaling). Temporal call parameters are given in milliseconds (ms), as range (followed by mean ± standard deviation, and number of analysed units in brackets).

Cophyla berara **n.sp.**, Figs 1–3, Tables 1 & 2

Holotype. ZSM 410/2000, adult male (Fig. 1), collected by F. Andreone, J.E. Randrianirina and M. Vences on 18 February 2000 at a site locally called Berara, located within the Anabohazo forest, Sahamalaza Peninsula, northwestern Madagascar (14°18.55′S, 47°54.92′E, 170 m above sea level).

Paratypes. MRSN A2502-A2506, five adult males, collected by F. Andreone, J.E. Randrianirina and M. Vences at the type locality, between 13 and 23 February 2000.

Diagnosis and comparisons. A microhylid frog assigned to the genus *Cophyla* in the subfamily Cophylinae because of its morphological similarity and molecular phylogenetic relationships to the

Table 1. Morphometric measurements (in mm) of the holotype and paratypes of *Cophyla berara*, holotype of *Platypelis occultans* and two specimens of *Cophyla phyllodactyla.*

Catalogue number	Status	Sex	SVL*	HW	HL	TD	ED	END	NSD	NND	HAL	FORL	HIL	FOTL	FOL
Cophyla berara															
ZSM 410/2000	HT	M	24.4	7.7	7.8	1.5	2.5	2.0	1.1	2.3	6.3	12.5	32.0	14.1	8.9
MRSN A2502	PT	M	25.8	7.0	7.2	1.6	2.7	2.1	1.1	2.4	5.8	13.6	33.5	14.6	9.1
MRSN A2503	PT	M	22.8	6.7	6.6	1.4	2.6	1.6	0.9	2.4	5.5	11.9	29.7	13.5	8.5
MRSN A2504	PT	M	22.6	6.8	6.9	1.2	2.4	1.8	0.9	2.1	5.2	12.0	29.5	12.9	9.2
MRSN A2505	PT	M	26.2	7.1	7.2	1.2	2.7	2.0	1.0	2.3	6.2	13.0	32.0	14.4	9.0
MRSN A2506	PT	M	24.0	7.1	7.1	1.3	2.5	2.0	1.0	2.4	6.0	12.6	32.6	14.4	9.5
Cophyla phyllodactyla (Nosy Be)															
ZSM 460/2000	–	M	27.4	9.0	8.3	1.3	3.0	2.0	1.3	2.5	8.0	16.1	37.9	17.5	11.2
Cophyla phyllodactyla (Manongarivo)															
ZSM 808/2003	–	M	27.9	9.3	7.7	1.4	2.8	2.1	1.4	2.5	7.5	15.5	36.3	17.3	11.0
Platypelis occultans (Nosy Be and Marojejy)															
ZFMK 53735	HT	M	21.0	6.4	6.4	1.0	2.4	1.5	1.0	2.0	5.0	11.6	25.7	11.7	7.3
MRSN A2660	–	M	20.0	5.7	6.1	1.0	2.4	1.5	1.0	2.2	5.3	12.4	25.9	11.5	7.0

*For abbreviations of measured variables, see Materials and Methods; other abbreviations: M (male), HT (holotype), PT (paratype).

type species of this genus, *Cophyla phyllodactyla.* Among other arboreal species of cophylines, the new species differs from *Plethodontohyla mihanika* Vences, Raxworthy, Nussbaum & Glaw, 2003, *P. notosticta* (Günther, 1877) and *P. inguinalis* Boulenger, 1882, by lacking dorsolateral folds (vs presence), and by numerous additional characters such as size and colouration; from species of *Anodonthyla* by lacking a distinct prepollex in males. *Cophyla berara* differs from most species of *Platypelis* by the combination of size (SVL 23–26 mm) and the very long note duration of advertisement calls (774–824 ms). Characters of *Platypelis* species that differ conspicuously from *C. berara* are as follows: *P. alticola* (Guibé, 1974) (SVL 38 mm), *P. barbouri* Noble, 1940 (note duration c. 160 ms), *P. cowani* Boulenger, 1882 (SVL of holotype 30 mm), *P. grandis* (Boulenger, 1889) (SVL 43–105 mm), *P. milloti* Guibé, 1950 (note duration 41–51 ms), *P. pollicaris* (Boulenger, 1889) (SVL of holotype 28 mm), *P. tuberifera* (Methuen, 1920) (SVL 30–40 mm). *P. tsaratananaensis* Guibé, 1974, is slightly larger (SVL 26–28 mm), has a much more elongated body, and its third toe is much shorter than its fifth toe (*vs* slightly shorter or of similar length). *Cophyla berara* differs from *C. phyllodactyla* by a smaller size (SVL of adult males 23–26 *vs* 27–29 mm) and by much longer note durations in its advertisement calls (774–824 ms *vs* 360–450 ms). *Platypelis occultans* Glaw & Vences, 1992, might be closely related to *C. berara* and might belong to the genus *Cophyla* (see discussion below) but differs by being smaller (SVL 18–21 *vs* 23–26 mm) and having a shorter note duration in its advertisement calls (325–550 ms).

Description of the holotype. Specimen in good state of preservation. Right forelimb amputated as tissue sample for genetic analysis. SVL 24.4 mm (for other measurements see Table 1). Body slender; head approximately as wide as long, not wider than body; snout slightly pointed in dorsal and lateral views; nostrils directed laterally, very slightly protuberant, nearer to tip of snout than to eye; canthus rostralis distinct, concave; loreal region concave; tympanum distinct, rounded, 60% of eye diameter; supratympanic fold rather indistinct, straight; tongue ovoid, relatively narrow, posteriorly free and not notched or forked; maxillary teeth present; vomerine teeth present as a minute central agglomeration; choanae rounded. Arm slender; distinct, single subarticular tubercle; a single large outer metacarpal tubercle; large inner metacarpal tubercle, forming distinct protuberance at prepollex; fingers without webbing; relative length of fingers 1<2=4<3, fourth finger of similar length as second; finger discs distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaching between forelimb and tympanum when hindlimb adpressed along body; tibia length (9.8 mm) 33% of SVL; lateral metatarsalia strongly connected; small inner metatarsal tubercles present, outer metatarsal tubercle not clearly recognizable; distinct traces of webbing between toes; relative

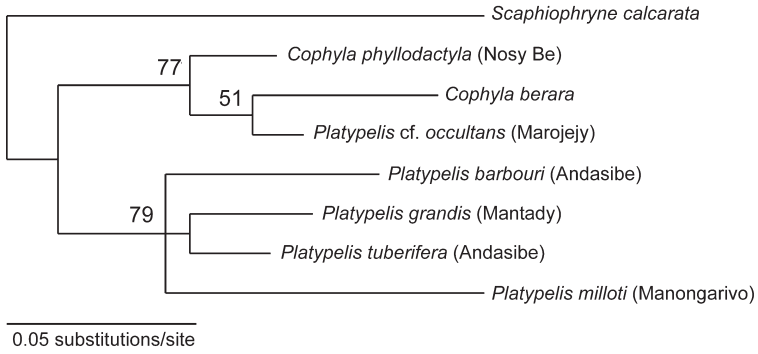

Fig. 2. Maximum likelihood phylogram based on analysis of 446 base pairs of a fragment of the mitochondrial 16S rRNA gene (after exclusion of a hypervariable region and gapped positions). The analysis was performed under a Tamura-Nei substitution model with empirical base frequencies and substitution rates, a gamma shape parameter of 0.4960 and a proportion of invariable sites of 0.5023, as selected by Modeltest (Posada & Crandall 1998). Numbers are values of a bootstrap analysis (500 replicates) as a percentage. *Scaphiophryne calcarata* was used as the outgroup.

length of toes 1<2<5=3<4; third toe as long as length or slightly shorter than fifth. Skin on dorsum smooth, without dorsolateral folds. Ventral skin smooth on throat, slightly granular on chest and belly.

After three years in preservative, dorsum light brown with indistinct darker markings. A dark hourglass-marking is present on the posterior head. Dark brown colour bordering ventrally the supratympanic fold. The ventral side is uniformly cream. In life (Fig. 1) the dorsum was light yellowish brown, with some yellowish pigment on the flanks. The belly was whitish. The iris was metallic light brown; the outer iris area was grey with a blue-greenish shade.

Variation. The five paratypes are morphologically very similar to the holotype (Table 1). In most paratypes, the third toe is shorter than the fifth toe, while MRSN A2504 is similar to the holotype in having these two toes of similar length. The tibiotarsal articulation reaches to the forelimb insertion in most specimens, while MRSN A2506 is similar to the holotype in that the tibiotarsal articulation reaches between forelimb insertion and tympanum.

Etymology. The specific name is derived from the type locality, the Berara forest. It is used as noun in the nominative singular, standing in apposition to the generic name.

Molecular phylogenetic relationships. The 16S rDNA fragment sequenced had a length of *c.* 550 base pairs. After exclusion of a central hypervariable region that could not be unambiguously aligned, and of all gapped positions, 446 characters were available for analysis. Of these, 343 were constant and 49 were parsimony-informative. The maximum likelihood analysis yielded the tree presented in Fig. 2; four of the included species of *Platypelis,* which had been selected in order to represent the variety in this genus, formed a monophyletic group to the exclusion of *Cophyla berara, C. phyllodactyla* and *Platypelis* cf. *occultans.* In the latter clade, *C. berara* was the sister group of *P.* cf. *occultans.* In the complete DNA fragment, uncorrected pairwise sequence divergence was 5.9% between *C. berara* and *P.* cf. *occultans,* and 8.2% between *C. berara* and *C. phyllodactyla.*

Natural history. Males of *Cophyla berara* were observed calling from leaves of bushes or trees at heights of 1–2 m. Calls were heard only at night, and calling specimens were very common at some sites in the forest. However, they did not form distinct breeding aggregations and calls were not emitted from positions around free water bodies. It can therefore be assumed that this species breeds in tree holes and similar water-filled cavities, as it is known for *Cophyla phyllodactyla* and numerous species of *Platypelis* (Blommers-Schlösser 1975; Glaw & Vences 1994).

Advertisement call. Recorded on 19 February 2000 at 23.5°C air temperature. The call consists of a single melodious note that is repeated after regular intervals. Calls were heard and recorded from numerous individuals that all agreed in temporal and spectral call characteristics. The following description is based on calls of one individual. The frequency is 2800–3000 Hz, in those recordings made from a close distance (Fig. 3) a harmonic is sometimes visible at 8700–8800 Hz. Note repetition rate is about 0.5 per

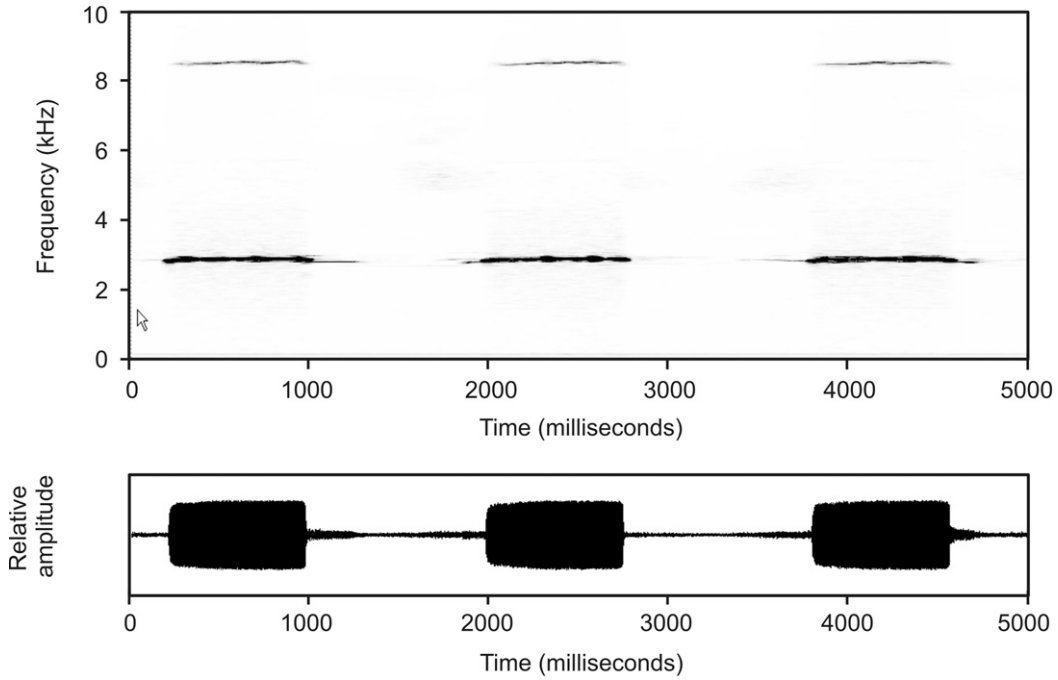

Fig. 3. Sonagram and oscillogram of the advertisement call of *Cophyla berara*, recorded at the type locality at 23.5°C air temperature.

second. Note duration is 774–824 ms (795.1 ± 13.4 ms, $n = 10$), duration of intervals between notes is 986–2272 ms (1318 ± 450 ms, $n = 10$).

Bioacoustic comparisons. Table 2 summarizes call data for *Cophyla berara* as presented herein, literature data on the calls of *Cophyla phyllodactyla* and *Platypelis occultans* (from Glaw & Vences 1992, 1994), as well as new information of the call of the latter species from Sambava and Marojejy. According to these data, *Cophyla berara* is characterized by exceptionally long notes that have never been recorded in any population of the other two species and in no other cophyline microhylid species either.

DISCUSSION

Until recently, *Cophyla* has been considered to be a monotypic genus restricted to Nosy Be in northwestern Madagascar (Blommers-Schlösser & Blanc 1991). Glaw & Vences (1992) provided the first observations on the natural history of the only known species, *C. phyllodactyla,* and reported

Table 2. Summary of temporal and spectral characteristics of advertisement calls of *Cophyla berara*, *C. phyllodactyla** and *Platypelis occultans* from various localities.

Species	Locality	Recording date and conditions	Note duration (ms)	Interval duration (ms)	Frequency (Hz)
Cophyla berara	Berara	19 Feb 2000, 23.5°C	774–824 ($n = 10$)	986–2272 ($n = 10$)	2800–3000
Cophyla phyllodactyla	Nosy Be	19 Jan 1992, 26°C	360–450	555–605	2300–2500
Platypelis occultans	Nosy Be	22 Jan 1992, 26°C	500–550	1210–1360	4000
Platypelis occultans	Marojejy	22 Feb 1995, 25°C	411–475 ($n = 35$)	1126–1994 ($n = 32$)	3450–3950
Platypelis occultans	Sambava 1	18 Feb 1995, 29°C	325–393 ($n = 20$)	837–1219 ($n = 16$)	3100–3300
Platypelis occultans	Sambava 2	23 Mar 1991	390–450	730–850	3000
Platypelis occultans	Voloina	19 Mar1991	*c.* 400	1600–1900	5500

*Data for *Cophyla phyllodactyla* and *Platypelis occultans* from Nosy Be, Voloina and Sambava 2 are from Glaw & Vences (1994) and refer to calls of one individual each (number of notes and intervals measured not available). Data for *Cophyla berara* and *Platypelis occultans* from Marojejy and Sambava 1 were newly analysed in this study (one individual of *C. berara*, range from measurements of three individuals each in the two *P. occultans* populations).

on a specimen from Sambava in northeastern Madagascar that possibly belongs to this genus and species. However, the relationships of *Cophyla,* and its validity next to the species-rich genus *Platypelis* remained uncertain.

Molecular data as presented above and in Andreone *et al.* (2004) indicated that *Cophyla* is genetically highly differentiated, with placement as a sister-group of *Platypelis.* While Andreone *et al.* (2004) analysed sequences of only *Cophyla berara* (as *Cophyla* sp.), we included molecular data also for *C. phyllodactyla,* demonstrating the close relationship of these two species. Hence, at least two species of *Cophyla* are known at present, and these are biogeographically restricted to northwestern, possibly also northeastern Madagascar. However, the generic assignment of these taxa, and the diagnoses of *Platypelis* and *Cophyla,* are in need of revision and should include those osteological characters (reduction of vomer and clavicle) that are thought to be diagnostic for *Cophyla* (Blommers-Schlösser & Blanc 1991).

The number of species in *Cophyla* may yet increase if *Platypelis occultans* is actually demonstrated to belong to this genus. So far we have not been able to obtain genetic data for *P. occultans* from its type locality Nosy Be. The DNA sequence data here tentatively assigned to this species comes from a specimen (MRSN A2660) from northeastern Madagascar that agrees in body size and general morphology with *P. occultans.* It was collected at Marojejy (about 600 m a.s.l.) by J.E. Randrianirina. No call of this individual was recorded, although calls of other Marojejy specimens agree with those from Nosy Be (Table 2).

Cophyla berara has so far only been recorded from Sahamalaza Peninsula. This area has recently received legal protection, based on its transitional forests and a number of unique faunal elements. It was recently designated as a Marine Protected Area, deserving the status of UNESCO Man and Biosphere Reserve (Projet ZICOMA, 1999; Andreone 2003; Anon. 2003). Our surveys in other reserves and forests in northwestern Madagascar (Lokobe, Tsaratanana, Manongarivo) did not yield any records of this species. On the eastern slopes of Manongarivo Special Reserve we collected *Cophyla phyllodactyla* which is also widespread at Nosy Be and in the Lokobe Strict Nature Reserve (Andreone *et al.* 2003). We suspect that *Cophyla berara* also occurs at other sites (e.g. on the western slopes of Manongarivo), but it is likely to be a localized endemic of northwestern Madagascar. Since *C. phyllodactyla* is usually found in anthropogenic habitats (Glaw & Vences 1994), we suspect that *C. berara* is present in some traditional coffee and cacao plantations as well. Nevertheless, like other cophylines (Andreone & Luiselli, 2003), this new *Cophyla* might be quite sensitive to habitat alteration. Evidence for its occurrence outside primary forest (e.g. in plantations) needs to be confirmed. We therefore suggest that this species might be vulnerable and its distribution area, habitat requirements and conservation status therefore require further investigation.

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