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A new phytotelmic species of *Platypelis* (Microhylidae: Cophylinae) from the Betampona Reserve, eastern Madagascar

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Abstract. We describe a new arboreal and diminutive species of the genus *Platypelis* from the Réserve Naturelle Intégrale N. 1 de Betampona, one of the last low-altitude rainforest fragments of eastern Madagascar. *P. karenae* sp. nov. is a phytotelmic species, living among leaves of *Pandanus* spp. and those of a herbaceous plant of the genus *Crinum*. Amongst species of comparable size, the new species is most similar to *P. tetra*, with which it shares a similar life history of occupying leaf axils of phytotelms. Phylogenetically, *P. karenae* is sister to *P. tuberifera* yet differentiated by a high level of genetic divergence (>7% p-distance for the analysed fragment of the 16S rRNA gene), its distinctly smaller size, acoustic repertoire, and colour pattern. The mitochondrial, nuclear, bioacoustic, and morphological data all independently support the validity of this new species.

Key words. Amphibia, Anura, new species, miniaturisation, *P. karenae* sp. n.

Introduction

Frogs of the highly diverse family Microhylidae are distributed throughout the tropical forests of the globe, and exhibit a wide variety of habitat adaptations and morphological variations. The cophyline genus *Platypelis* is endemic to Madagascar, and currently includes 12 described species and at least other six confirmed candidate species (Glaw & Vences 2007, Vieites et al. 2009, Perl et al. 2014). The species of this genus are small to medium-sized frogs (16–40 mm), with the exception of *P. grandis,* which reaches up to 105 mm in snout–vent length (SVL) and is one of the largest microhylid frogs worldwide (Glaw & Vences 2007).

All known *Platypelis* species are arboreal, with enlarged finger and toe disks, and breed in phytotelmata or water-filled bamboo internodes or tree holes. As far as is known, the male guards the eggs and non-feeding tadpoles until their metamorphosis (Blommers-Schlösser 1975). In terms of biogeography, the genus is mainly distributed in the rainforests of northern and northeastern Madagascar, where several species have recently been discovered and from where several confirmed candidate species are currently known and still need to be formally described (Andreone et al. 2005, Glaw & Vences 2007, Rakotoarison et al. 2012, Rosa et al. 2012). No species of *Platypelis* has yet been found in the arid western regions and the deciduous forests of Madagascar, presumably because of their need for continuously high humidity (Mercurio et al. 2008, Bora et al. 2010) and appropriate phytotelmic breeding sites.

Typically, *Platypelis* males call perched on leaves or branches and inflate a single, quite expandable, subgular vocal sac. Their vocalizations show a rather similar temporal succession, consisting of single and monotonous notes repeated in almost endless series, as in most cophylines (Glaw & Vences 2007). Notable exceptions to this pattern

are *P. grandis*, whose males hide inside tree holes or *Ravenala* palm trees and emit resonating and abrupt, inharmoniously modulated call pulses at rather irregular intervals (Blommers-Schlösser 1975), and *P. tsaratananaensis*, which emits double-note calls (Rakotoarison et al. 2012).

Anurans are notable among vertebrate groups for the extraordinary diversity of their reproductive modes (Duellman 1985). Occupation of phytotelmata is a widely followed strategy among amphibians, and has originated as an ecological innovation multiple times convergently (e.g., Lehtinen 2002, Andreone et al. 2010). In Madagascar, plants such as *Pandanus* spp. retain rainwater in their leaf axils (at least during the rainy season), thus providing frogs with suitable habitats for breeding, resting and feeding activities. Most species of the mantelline subgenus *Pandanusicola* (Lehtinen et al. 2004), the recently described *Blommersia angolafa* (Andreone et al. 2010), and the majority of the *Plethodontohyla*, *Anodonthyla*, *Cophyla* and *Platypelis* species rely on phytotelmata, dwelling in these water-holding plants throughout all or a great part of their life cycle (Andreone et al. 2003, Glaw & Vences 2007, Lehtinen 2002). Non-feeding tadpoles develop inside these ephemeral small pools, benefiting from parental care (Blommers-Schlösser 1975, Andreone et al. 2005, Glaw & Vences 2007). This situation may confer some advantages, both in terms of safety and reduced competition (Summers & McKeon 2004).

In this paper, we describe a new diminutive *Platypelis* that breeds in phytotelmata of *Pandanus* spp. and a herbaceous plant of the genus *Crinum*. We also provide observations on the life history and assess the molecular phylogenetic relationships of the new taxon to other *Platypelis* species.

Material and methods

Study periods and site

All individuals of the new *Platypelis* species were found during herpetological survey works carried out at the Réserve Naturelle Intégrale (RNI) de Betampona in 2004, 2006, and 2007. This fragment of lowland rainforest is an integrated part of the protected area network in Madagascar (ANGAP 2003). Located on the northern central east coast of Madagascar between 17°15'–17°55' S and 49°12'–49°15' E (Britt et al. 2004), the reserve extends from an altitude of 275 to 650 m above sea level (Razokiny 1985).

The forest has been under government protection since 1927. Around 50% of Betampona is undisturbed primary rainforest, with an additional ~ 35% being characterized as recovering primary rainforest and the remainder as secondary forest (Armstrong et al. 2011).

Field methods

As a standard procedure, frogs were searched for both during the day and night with the aid of headlamps and torchlights. Searches took into account all plant species that could potentially offer phytotelmata. Acoustic encounter surveys were used to detect calling males. The vocalizations of each individual were recorded with a Marantz PMD 660 digital recorder, accessorized with a semi-directional microphone. Live colour patterns were recorded by photography at the time of capture. A limited number of individuals were collected as museum vouchers. Specimens were euthanised (by an overdose of MS222 or chlorobutanol), a toe clipping was removed for genetic analyses, and specimens were then fixed in 10% buffered formalin or 90% ethanol (later transferred to 70% ethanol).

Morphometric measurements

The following morphological features were measured to the nearest 0.1 mm with a digital calliper: SVL (snout–vent length); HL (head length, diagonal straight distance between the maxillary commissure to the snout tip); HW (head width); ED (horizontal eye diameter); TD (horizontal tympanum diameter); END (eye–nostril distance); NSD (nostril-tip of the snout distance); FORL (forelimb length, from the inside of elbow to the tip of the longest finger); HAL (hand length, from the carpal-metacarpal articulations to the tip of the longest finger); HLL (hind limb length, from the cloaca to the tip of the longest toe); FOL (foot length, from the tarsal-metatarsal articulations to the tip of the longest toe).

Acronyms used are as follows: MRSN – Museo Regionale di Scienze Naturali, Torino, Italy; AMNH – American Museum of Natural History, New York, USA; ZSM – Zoologische Staatssammlung München, Germany; FAZC refers to field numbers of F. Andreone; ZCMV those of M. Vences; DRV to those of D. R. Vieites; ACZC to those of A. Crottini; PSG to those of P.-S. Gehring and FGZC to those of F. Glaw.

The type specimens were compared to the similarly sized *P. tetra* and the sister species *P. tuberifera* (Tab. 1) as follows: eight *P. tuberifera* specimens (MRSN A4729, A4748, A1888.1, A4728, A4733, A4737, A2946, and A6254); four specimens (holotype and paratypes) of *P. tetra* from Anjanaharibe-Sud (MRSN A2171–2174), two specimens from Lohanandroranga (AMNH A167267, 167276) and two specimens from Andramanalana (AMNH A181900, 181901) attributed to *P. tetra*.

Molecular analyses

Toe clips were collected from a selected number of individuals as tissue samples for molecular analysis. Eight samples of *P. karenae* sp. n. from Betampona Natural Reserve; one sample of *P.* sp. aff. *tetra* 1 from Masoala (Farankaraina) and two from near Makira (Ambodivohangy); one sample of *P.* sp. aff. *tetra* 2 from Ambodiriana; one sample of *P. tetra* from Lohanandroranga (near Bealanana) and 23 samples of *P. tuberifera* from Betampona (1 sample), Masoala (6),

Table 1. Morphometric measurements (in mm) of specimens of *Platypelis karenae* sp. n., *P. tuberifera*, and *P. tetra*. M – male, F – female, HT – holotype, PT – paratype. For other abbreviations, see the running text.

Museum number	Types	Provenience	Sex	SVL	HL	HW	ED	TD	END	NSD	FORL	HAL	HLL	FOL
P. karenae sp. n.														
MRSN A6847	HT	Betampona	M	17.4	6.2	5.6	2.0	1.1	2.2	1.6	6.6	3.5	20.0	6.1
MRSN A5686	PT	Betampona	M	16.1	6.5	6.1	2.5	1.2	1.6	1.7	5.3	3.7	19.4	5.6
MRSN A6369	PT	Betampona	M	16.7	5.7	6.5	2.1	1.4	1.7	2.0	7.3	3.4	19.1	6.4
MRSN A6293	PT	Betampona	M	16.5	6.5	6.1	2.6	1.3	1.5	1.4	7.6	4.2	20.1	5.3
AMNH A173882	PT	Betampona	M	17.0	5.6	6.8	2.0	1.0	1.6	1.4	7.0	4.0	18.6	5.6
MRSN A6848	PT	Betampona	F	17.4	6.3	5.6	2.3	1.3	1.5	1.7	6.8	4.0	20.7	5.8
MRSN A5689	PT	Betampona	F	18.3	7.2	6.2	2.7	1.2	2.2	1.5	7.0	3.7	24.5	5.6
MRSN A5687	PT	Betampona	F	16.7	6.1	5.5	2.0	1.3	1.9	1.9	6.5	3.8	21.1	5.7
MRSN A6286	PT	Betampona	F	17.5	6.1	6.6	2.1	1.4	1.8	1.9	6.7	3.6	19.4	5.8
P. tuberifera														
MRSN A4729		Ambolokopatrika	M	30.6	13.2	12.5	4.1	2.6	2.9	3.0	11.4	7.7	39.3	11.6
MRSN A4733		Ambolokopatrika	M	34.6	11.9	13.3	3.9	2.3	3.4	3.0	14.9	8.8	42.1	13.5
MRSN A4737		Ambolokopatrika	M	29.1	11.5	11.8	3.7	2.0	2.3	2.5	10.7	7.7	38.8	10.7
MRSN A2946		Masoala	M	25.3	10.5	10.0	3.4	1.2	1.7	2.5	11.1	6.4	34.8	10.1
MRSN A4748		Masoala	F	38.3	13.7	14.1	5.1	2.7	3.6	3.4	14.2	8.1	41.7	13.5
MRSN A1888.1		Tsararano	F	34.8	12.7	12.9	4.1	2.7	3.2	3.6	16.1	9.3	48.2	14.5
MRSN A4728		Masoala	F	38.1	14.8	15.6	4.3	3.1	3.4	3.3	16.0	9.7	50.0	14.9
MRSN A6254		Betampona	F	34.7	12.3	12.5	4.1	16.0	3.5	3.1	12.2	7.1	39.5	10.8
P. tetra														
MRSN A2174	HT	Anjanaharibe-Sud	M	17.5	6.2	6.4	2.0	0.8	1.3	1.6	5.9	3.2	17.5	6.0
MRSN A2172	PT	Anjanaharibe-Sud	M	18.2	6.3	6.5	2.2	1.2	1.4	1.6	6.5	3.4	18.2	5.4
MRSN A2173	PT	Anjanaharibe-Sud	F	19.4	7.1	6.8	2.3	0.8	1.5	1.6	6.3	3.8	19.4	5.9
MRSN A2171	PT	Anjanaharibe-Sud	F	18.9	6.4	6.9	2.3	1.2	1.5	1.9	7.6	4.1	18.9	5.7
AMNH A181900		Andramanalana	F	16.8	5.4	6.3	1.9	0.6	1.4	1.2	6.1	3.3	19.0	5.5
AMNH A181901		Andramanalana	F	21.0	5.9	8.2	2.2	1.2	1.5	1.4	7.8	5.3	27.0	7.0
AMNH A167267		Lohanandroranga	F	23.1	5.1	8.6	2.1	1.2	1.4	1.6	8.0	6.0	29.0	7.5
AMNH A167276		Lohanandroranga	F	24.0	5.4	8.8	2.2	1.2	1.6	1.6	8.1	6.1	31.0	8.4

Vevembe (1), Ambalabe (1), Tsararano (1), Ambolokopatrika (10), Antara (1), Ranomafana (1), and Besariaka (1) were used for molecular analyses (see Tab. 2 for details). To prevent potential contamination, sampling was done using sterilized equipment (NSW National Parks and Wildlife Service 2001, Speare et al. 2004).

Total genomic DNA was extracted from the tissue samples using proteinase K digestion (10 mg/ml concentration) followed by a standard salt extraction protocol (Bruford et al. 1992). We amplified one mitochondrial and one nuclear fragment: the mitochondrial rrnL (large ribosomal RNA, or 16S rRNA gene), and the proopiomelanocortin gene (POMC). Standard Polymerase chain reactions were performed in a final volume of 11 µl and using 0.3 µl each of 10 pmol primer, 0.25 µl of total dNTP 10 mM (Promega), 0.08 µl of 5 U/ml GoTaq, and 2.5 µl 5X Green GoTaq Reaction Buffer (Promega). To sequence a fragment of ca 550 bp of the large ribosomal RNA gene, we used the primers 16SL3 5'-AGCAAAGAHYWWACCTCGTACCTTTTGCAT-3' and 16SAH 5'-ATGTTTTTGATAAACAGGCG-3' as described in Vences et al. (2003). To sequence the POMC fragment, we used the primers POMC DRVF1 5'-ATATGTCATGASCCAYTTYCGCTGGAA-3' and POMC DRVR1 5'-GGCRTTYTTGAAWAGAGTCATTAGWGG-3' (Vieites et al. 2007) as outlined in Vences et al. (2010). For the nuclear gene fragment, both strands of the successfully amplified PCR products, treated with ExoSAP-IT (USB) to inactivate remaining primers and dNTPs, were directly used for the cycle sequencing reaction, using dye-labelled terminators (Applied Biosystems) with the amplification primers. Labelled fragments were analysed on an ABI 3130 automated DNA sequencer (Applied Biosystems). Sequences were compared with GenBank sequences, and chromatographs were visually checked and edited, when necessary, using CodonCode Aligner (version 3.7.1, Codon Code Corporation).

Homologous 16S rRNA gene sequences of *P. pollicaris* (EU341098), *P. tuberifera* from Marojejy (EU352823) and Andasibe (EU341093), *P. barbouri* (AY594057), *P. alticola* (JX519434), *P. tsaratananaensis* (JX519457), *P. milloti* (EU341094), and *P. grandis* (EU341095) were retrieved

Table 2. List of tissue samples included in the present study for molecular analyses (ID), species identification, localities, latitude, longitude, and GenBank accession numbers (where available).

ID	Species	Locality	Campsite	GPS coordinates	16S	POMC
FGZC 180	*P. grandis*	Andohahela	Andohahela Camp 2	24°44'18.00"S, 046°50'25.00"E	EU341095	–
MRSN A5686	*P. karenae* sp. n.	Betampona	Sahabefoza	17°54'52.70"S, 049°12'30.40"E	KM817790	KM817818
MRSN A5687	*P. karenae* sp. n.	Betampona	Sahabefoza	17°54'52.70"S, 049°12'30.40"E	KM817791	KM817819
MRSN A5689	*P. karenae* sp. n.	Betampona	Sahabefoza	17°54'52.70"S, 049°12'30.40"E	KM817792	KM817820
MRSN A6286	*P. karenae* sp. n.	Betampona	Sahabefoza	17°54'52.70"S, 049°12'30.40"E	HM364748	KM817823
FAZC 13605	*P. karenae* sp. n.	Betampona	Sahambendrana	17°53'54.30"S, 049°12'55.30"E	HM364747	KM817822
MRSN A6369	*P. karenae* sp. n.	Betampona	Sahambendrana	17°53'53.40"S, 049°12'57.80"E	GU371305	–
MRSN A6847	*P. karenae* sp. n.	Betampona	Sahambendrana	17°53'54.50"S, 049°12'55.20"E	HM364746	KM817821
FAZC 13975	*P. karenae* sp. n.	Betampona	Vohitsivalana	17°53'17.80"S, 049°12'14.70"E	HM364749	KM817825
MRSN A6557	*P. tuberifera*	Ambalabe	–	19°08'13.47"S, 048°32'15.12"E	KM817806	KM817840
MRSN A4729	*P. tuberifera*	Ambolokopatrika	Andemakatsara	14°31'48.00"S, 049°26'30.00"E	KM817808	KM817843
MRSN A4730	*P. tuberifera*	Ambolokopatrika	Andemakatsara	14°31'48.00"S, 049°26'30.00"E	KM817809	KM817844
MRSN A4731	*P. tuberifera*	Ambolokopatrika	Andemakatsara	14°31'48.00"S, 049°26'30.00"E	KM817807	KM817842
MRSN A4732	*P. tuberifera*	Ambolokopatrika	Andemakatsara	14°31'48.00"S, 049°26'30.00"E	KM817810	KM817845
MRSN A4736	*P. tuberifera*	Ambolokopatrika	Andemakatsara	14°31'48.00"S, 049°26'30.00"E	KM817811	KM817846
MRSN A4737	*P. tuberifera*	Ambolokopatrika	Andemakatsara	14°31'48.00"S, 049°26'30.00"E	KM817812	KM817847
MRSN A4738	*P. tuberifera*	Ambolokopatrika	Andemakatsara	14°31'48.00"S, 049°26'30.00"E	KM817813	KM817848
MRSN A4733	*P. tuberifera*	Ambolokopatrika	Andranomadio	14°32'24.00"S, 049°26'18.00"E	–	KM817852
MRSN A4734	*P. tuberifera*	Ambolokopatrika	Andranomadio	14°32'24.00"S, 049°26'18.00"E	KM817814	KM817853
MRSN A4755	*P. tuberifera*	Ambolokopatrika	Andranomadio	14°32'24.00"S, 049°26'18.00"E	KM817815	KM817849
ZSM 2/2002	*P. tuberifera*	Andasibe	–	no coordinate available	EU341093	–
MRSN A5025	*P. tuberifera*	Antara region	Sahavontsira	16°53'14.00"S, 049°10'58.00"E	KM817816	KM817850
MRSN A4758	*P. tuberifera*	Besariaka	Ambinaninimiakamidina	14°50'48.00"S, 049°35'42.00"E	KM817802	KM817836
MRSN A6254	*P. tuberifera*	Betampona	Sahambendrana	17°54'04.70"S, 049°12'52.40"E	KM817798	KM817831
ZSM 352/2005	*P. tuberifera*	Marojejy	Camp Simpona	14°26'11.94"S, 049°44'36.06"E	EU352823	–
MRSN A2860	*P. tuberifera*	Masoala	Amparihy	15°25'03.60"S, 049°56'25.20"E	KM817804	KM817837
MRSN A4728	*P. tuberifera*	Masoala	Andasin'i Governera	15°18'33.00"S, 050°01'24.00"E	KM817799	KM817833
MRSN A4748	*P. tuberifera*	Masoala	Antsarahan'Ambarato	15°19'03.51"S, 050°04'06.38"E	KM817801	KM817835
MRSN A4735	*P. tuberifera*	Masoala	Beanjada	15°19'13.06"S, 050°06'43.67"E	KM817800	KM817834
MRSN A4725	*P. tuberifera*	Masoala	Menamalona	15°22'52.20"S, 049°59'16.20"E	KM817805	KM817838
FAZC 15211	*P. tuberifera*	Masoala	Tampolo	15°43'59.94"S, 049°57'31.02"E	KM817803	KM817851
ACZC 1762	*P. tuberifera*	Ranomafana	–	no coordinate available	KM817817	–
MRSN A1888	*P. tuberifera*	Tsararano	Antsarahan'ny Tsararano	14°54'40.00"S, 049°41'23.00"E	–	KM817841
MRSN A6461	*P. tuberifera*	Vevembe	Vevembe	22°47'41.17"S, 047°11'13.67"E	–	KM817839
PSG 2202	P. sp. aff. *tetra* 1	Makira	Ambodivohangy	15°17'23.80"S, 049°37'13.00"E	KM817794	KM817827
PSG 2362	*P.* sp. aff. *tetra* 1	Makira	Ambodivohangy	15°17'23.80"S, 049°37'13.00"E	KM817795	KM817828
FAZC 14222	*P.* sp. aff. *tetra* 1	Masoala	Farankaraina	15°26'00.00"S, 049°51'00.00"E	KM817796	KM817829
ZCMV 8962	*P.* sp. aff. *tetra* 2	Ambodiriana	Ambodiriana forest	16°40'28.40"S, 049°42'10.00"E	KM817797	KM817830
ZSM 348/2005	*P. pollicaris*	Ambohitantely	Ambohitantely	18°11'58.02"S, 047°16'51.18"E	EU341098	–
MRSN A2616	*P. barbouri*	Besariaka	Ambinaninimiakamidina	14°49'18.00"S, 049°03'15.00"E	AY594057	–
ZSM 817/2003	*P. milloti*	Manongarivo	Camp 0	13°58'32.00"S, 048°25'36.00"E	EU341094	–
AMNH A167267	*P. tetra*	Bealanana region	Lohanandroranga	14°24'59.00"S, 048°08'51.00"E	KM817793	KM817826
DRV 6113	*P. alticola*	Tsaratanana	Antevialambazaha	14°10'26.87"S, 048°56'42.76"E	JX519434	–
ZCMV 12610	*P. tsaratananaensis*	Tsaratanana	Matsabory Maiky	14°09'09.22"S, 048°57'26.21"E	JX519457	–

from GenBank and added to the rrnL gene fragment alignment for outgroup rooting in the phylogenetic analyses. The purpose of the presented phylogenetic analyses is to show the closest phylogenetic relationship of the new species to *P. tuberifera* rather than to the morphologically similar *P. tetra*. For this reason, some distantly related described (e.g., *P. olgae, P. cowani*) and undescribed species of *Platypelis* are not included.

This alignment required the inclusion of gaps to account for indels in only a few cases. All newly determined sequences have been deposited in GenBank (accession numbers are provided in Tab. 2).

Uncorrected pairwise distances (p-distance transformed into percentage using the complete deletion option) amongst individuals of the same species and between analysed *Platypelis* species were computed using MEGA, version 6.06 (Tamura et al. 2013). We performed maximum likelihood (ML) and Bayesian inference searches of the mitochondrial 16S rRNA gene fragment. Four independent ML analyses were carried out in Treefinder (Jobb 2011), including the determination of the best substitution model based on the corrected Akaike Information Criterion (AIC). Support for the resulting ML topologies was obtained from bootstrap analyses as incorporated in Treefinder, with 1,000 replicates, 10 random addition sequences replicates, and TBR branch swapping. Bayesian analyses were conducted in MrBayes 3.2.1 (Ronquist et al. 2012). The GTR+I+G model was determined by AIC in jModelTest2 (Darriba et al. 2012) as the best-fitting model of substitution. We performed two runs of 10 million generations (started on random trees) and four incrementally heated Markov chains (using default heating values), sampling the Markov chains at intervals of 1,000 generations. Stabilization and convergence of likelihood values were checked by visualizing the log likelihoods associated with the posterior distribution of trees in the software Tracer (Rambaut & Drummond 2007), and occurred after about 3.5 million generations. The first four million generations were conservatively discarded, and six million trees were retained post burn-in and summed to generate the majority rule consensus tree.

Haplotypes of POMC data were inferred using the PHASE algorithm (Stephens et al. 2001) incorporated in the DnaSP software (Version 5.10.3, Librado & Rozas 2009). Haplotype network reconstruction of phased sequences of the POMC fragment was performed using the software TCS, version 1.21 (Clement et al. 2000). This software employs the method of Templeton et al. (1992) and calculates the number of mutational steps by which pairwise haplotypes differ, computing the probability of parsimony for pairwise differences until the probability exceeds 0.95 (no manual adjustment of the threshold was necessary).

Bioacoustic analyses

Calls were analysed with the acoustic software Adobe Audition 3.0 (as described in Rosa & Andreone 2010, Rosa et al. 2010), and compared to the existing database of frog vocalizations (Vences et al. 2006). Recordings were resampled at 44.1 Hz and 16-bit resolution in the mono pattern and in "Waveform" extension. Frequency information was obtained through Fast Fourier Transformation (FFT. width 1,024 points); the audio spectrogram was obtained in the Hanning window function with a resolution of 256 bands. Temporal measurements are provided as range, followed by mean, standard deviation, and the number of analysed units (n): analysed notes, calls or intervals.

Results

Platypelis karenae sp. n.

Figs 3–5

Remarks: This species has been previously regarded as a candidate species and referred to as *P.* sp. aff. *tetra* [Ca FJ559288] by Rosa et al. (2011) and Rosa et al. (2012), *P.* sp. by Andreone & Randriamahazo (2008), and *P.* sp. 2 by Vieites et al. (2009).

Holotype: MRSN A6847, adult male (ethanol-fixed and sequenced for DNA comparison) collected by G. M. Rosa, J. Noël & F. Andreone on 6 February 2007 at Sahambendrana campsite, 17°53'54.5" S, 049°12'55.2" E, 458 m a.s.l., Betampona, Region Atsinanana, Commune Rurale de Sahambala, east Madagascar.

Paratypes: All paratypes were collected at Betampona (same administrative data as for the holotype): AMNH A173882, adult male (formalin-fixed), collected by N. Rabibisoa, M. Randriambahiniarime and A. Ranjanaharisoa on 20 March 2004 at Sahambendrana campsite, 17°53'54.2" S, 49°12'55.4" E, 450 m a.s.l.; MRSN A5686, adult male, and MRSN A5687 and A5689, adult females (formalin-fixed and sequenced for DNA comparison), collected by F. Andreone on 8 February 2006 at Sahabefoza campsite, 17°54'52.7" S, 49°12'30.4" E, 343 m a.s.l.; MRSN A6286, adult female (ethanol-fixed and sequenced for DNA comparison), collected by G. M. Rosa and J. Noël on 6 March 2007 at Sahabefoza campsite, 17°54'52.7" S, 49°12'30.4" E, 343 m a.s.l.; MRSN A6293, adult male (ethanol-fixed), collected by G. M. Rosa and J. Noël on 8 February 2007 at Sahambendrana campsite, 17°53'53.8" S, 49°12'57.2" E, 393 m a.s.l.; MRSN A6369, adult male (ethanol-fixed and sequenced for DNA comparison), collected by G. M. Rosa, J. Noël and F. Andreone on 5 November 2007 at Sahambendrana campsite, 17°53'53.4" S, 49°12'57.8" E, 351 m a.s.l.; MRSN A6848, adult female (ethanol-fixed), collected by G. M. Rosa, J. Noël and F. Andreone on 6 February 2007 at Sahambendrana campsite, 17°53'54.5" S, 49°12'55.2" E, 458 m a.s.l.; MRSN A6977, three eggs (formalin-fixed) and MRSN A6978, three eggs (formalin-fixed), collected by J. Noël on 11 December 2010, at Sahambendrana campsite; MRSN A6979 and A6980, six tadpoles (ethanol-fixed), collected by J. Noël on 30 November 2010 at Maintimbato campsite, 17°53'47.5" S, 49°13'35.5" E, 270 m a.s.l.

Etymology: The species is dedicated to Karen L. M. Freeman, former Madagascar Fauna and Flora Group field coordinator and current research coordinator, in recognition of her dedication to the conservation of the Betampona rainforest.

Diagnosis: An arboreal and phytotelmic diminutive *Platypelis* species characterized by the following combination of characters: small adult size (SVL 16–18 mm); toe and

finger pads expanded, almost circular (rather than ovoid) in shape; hands and feet without webbing; tarso-metatarsal articulation reaching the tympanum; dorsal surface smooth; dorsal colour light yellow-light greenish with a wide brownish black dorsolateral stripe running from the eye to behind the axilla. The new species is assigned to the genus *Platypelis* based on its enlarged terminal finger discs, absence of finger-like prepollex, and molecular phylogenetic relationships (Wollenberg et al. 2008).

Comparison with other species: Within the *Platypelis* clade, the new species is close to *P. tuberifera*, sharing a very similar morphological appearance, including a wide dark stripe running from the posterior edge of the eye to beyond the axilla. However, *P. tuberifera* differs by its distinctly larger size (SVL of adult males up to 40 mm vs. up to 17 mm) and often-spotted pattern on the back vs. a uniformly light yellowish pattern on the dorsum, with a few or no lighter spots. The new species is distinguished from *Platypelis alticola*, *P. grandis*, *P. mavomavo*, *P. milloti*, *P. pollicaris*, and *P. tsaratananaensis* by its distinctly smaller size.

Among species of comparable size, *P. karenae* is most similar to *P. tetra*, with which it shares a similar life history of occupying leaf axils phytotelms (both have been found in *Pandanus* plants, but only *P. karenae* in herbaceous *Crinum* plants). *Platypelis karenae* differs from *P. tetra* by: (1) its colour pattern: *P. tetra* presents a much darker body colour pattern, with brownish markings and speckling; the legs have irregular tan spots and the dorsal body has at least four large whitish spots; (2) by the third toe being slightly shorter than the fifth (vs. third toe longer); and (3) by a completely smooth dorsum (vs. tuberculated).

Additionally, *P. karenae* differs from the other described *Platypelis* species as follows: from *P. barbouri*, *P. milloti*, *P. mavomavo*, *P. olgae*, and *P. ravus* by a uniform whitish ventral surface (vs. reddish or yellow colour on venter and/or ventral side of hind limbs); from *P. barbouri*, *P. grandis*, and *P. mavomavo* by a completely smooth dorsum (vs. tuberculated); from *P. barbouri* by the smoothness of the skin and presence of a wide dark dorsolateral stripe; from *P. milloti* by its pale yellow body colouration; and from *P. mavomavo,* by the third toe being slightly shorter than the fifth (vs. third toe longer). *P. cowani* was not taken into consideration due to the still unclear identification of the specimens and call of this species.

P. karenae is also distinct from all other species of *Platypelis* by its call, and further distinguished by molecular relationships and genetic divergence (see below). The similarly small-sized species *P. tetra* is placed with high support into a different clade (Figs 1–2).

Platypelis karenae also differs from other superficially similar microhylids in Madagascar by the following features: from *Anodonthyla* males (similar SVL) by the ab-

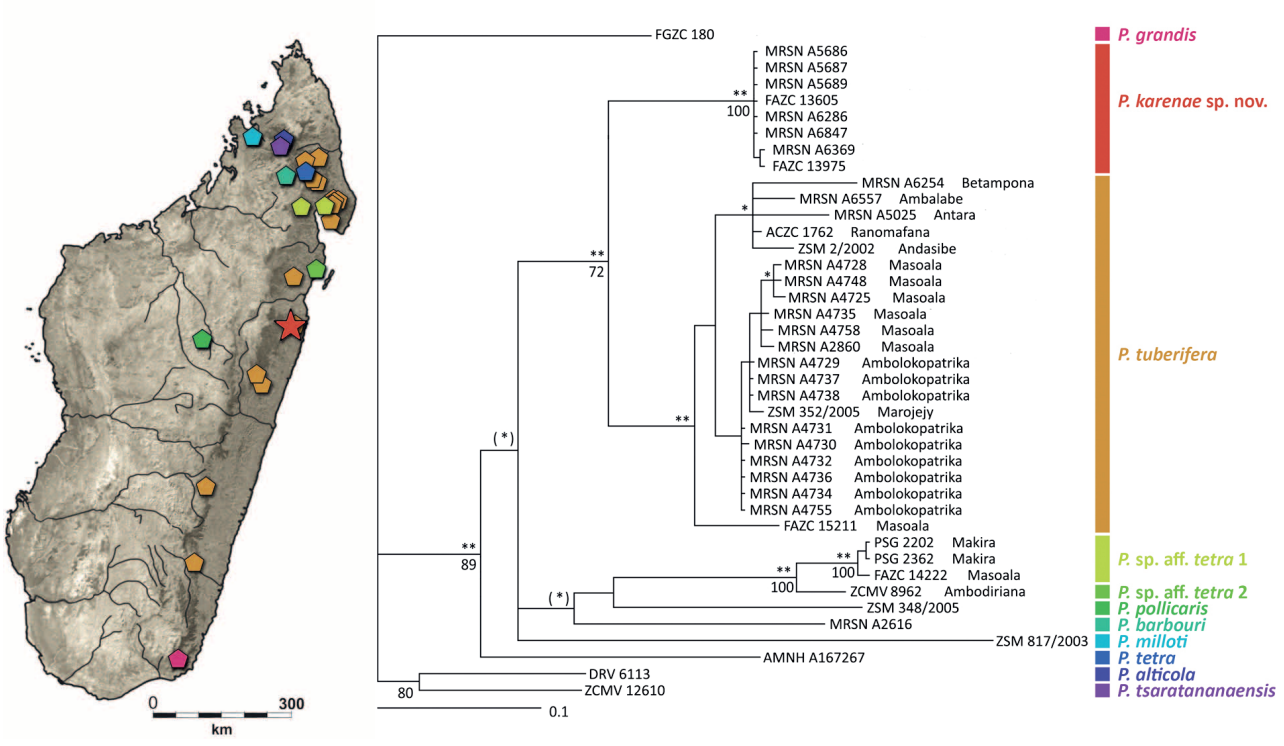

Figure 1. Bayesian inference tree of *Platypelis* species based on 586 bp of the mitochondrial 16S rRNA gene. Values at nodes are bootstrap values in percent from ML analyses; values below 70% are not shown. Asterisks denote Bayesian posterior probabilities values: * – 90–98%; * – 99%; ** – 100%. The distribution of the sampling localities across Madagascar is also indicated. The star points out Betampona, the type locality of *Platypelis karenae* sp. n.

sence of a distinct prepollex in males; and from *Cophyla phyllodactlya*, *C. occultans*, and *C. berara* by colouration (light yellowish vs. brownish) and call characteristics.

Description of the holotype: Adult male in a good state of preservation; snout–vent length 17.4 mm; for other measurements see Tab. 1. Body moderately stout; head wider than long, not wider than body; snout rounded in dorsal and lateral views; nostrils directed laterally, not protuberant, nearer to tip of snout than to eye; canthus rostralis indistinct; loreal region plain; tympanum rather indistinct, supratympanic fold distinct, straight; tongue ovoid, broad, free posteriorly and notched very slightly; maxillary teeth present; vomerine teeth not recognizable; choanae rounded. The single vocal sac is moderately expandable. Forelimb slender; subarticular tubercles single, indistinct; outer metacarpal tubercle large but low (not prominent); inner metacarpal tubercle moderately large, forming a protuberance at base of first finger; hand without webbings; fingers distinctly flattened and relatively broad throughout their lengths; relative lengths of fingers 1<2=4<3, fourth finger of similar length as second; finger disks distinctly enlarged, rounded; nuptial pads absent. Hind limbs slender; tibiotarsal articulation reaching tympanum when hind limb is adpressed along body; lateral metatarsals strongly connected; inner metatarsal tubercle small; outer metatarsal tubercle absent; no webbings between toes; toes flattened and relatively broad throughout their lengths; relative lengths of toes 1<2<3≤5<4; third toe slightly shorter than the fifth; toe disks distinctly enlarged, rounded. Dorsal skin smooth without dorsolateral folds. Ventral skin slightly granular on throat, and moderately granular on chest and belly.

In life, the holotype was uniformly light yellow with a few lighter spots on the dorsum, while a wide blackish brownish stripe extended from the posterior edge of the

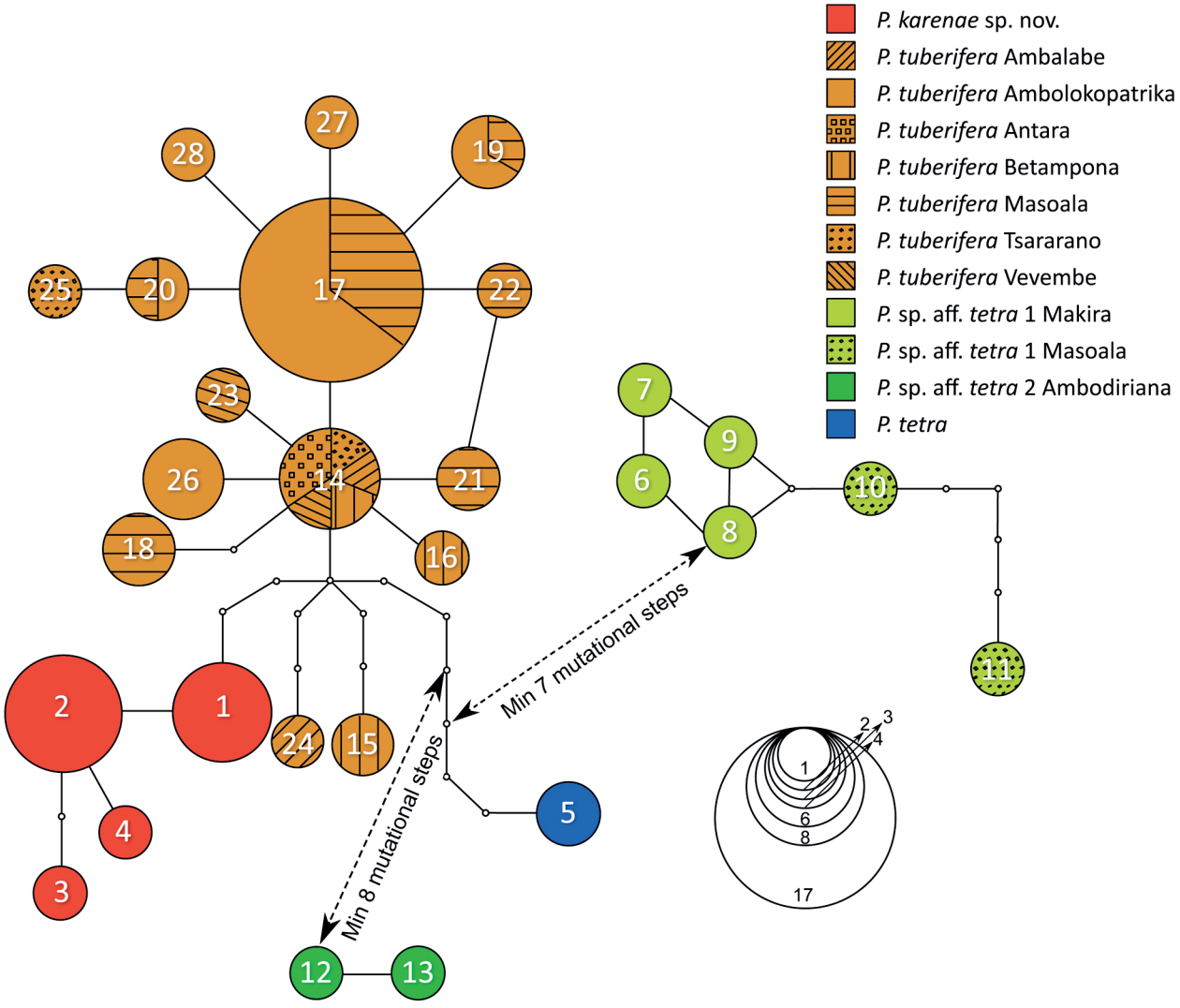

Figure 2. Haplotype network reconstruction of the nuclear POMC gene fragment in *P. karenae* sp. n., *P. tetra, P. tuberifera*, *P.* sp. aff. *tetra* 1, and *P.* sp. aff. *tetra* 2.

eye to a point around 0.5 mm behind the insertion point of the forearm, where it disintegrated into isolated dark spots. The iris was light brown – with darker scattered dots. The belly was off-white, and the vocal sac, during the emission of calls, translucent and bluish. After seven years in preservative, the holotype has become greyish, but the dorsolateral stripes are still evident.

Variation: The adult paratypes are similar to the holotype in general morphology and colour pattern; for measurements see Tab. 1. Females lack a vocal sac. Juveniles differ from adults by presenting more greenish body colouration with a larger number of scattered light spots on the dorsum, flanks, and limbs, and by having a reddish-brownish snout tip and iris (Fig. 3E). The dark wide lateral stripe is

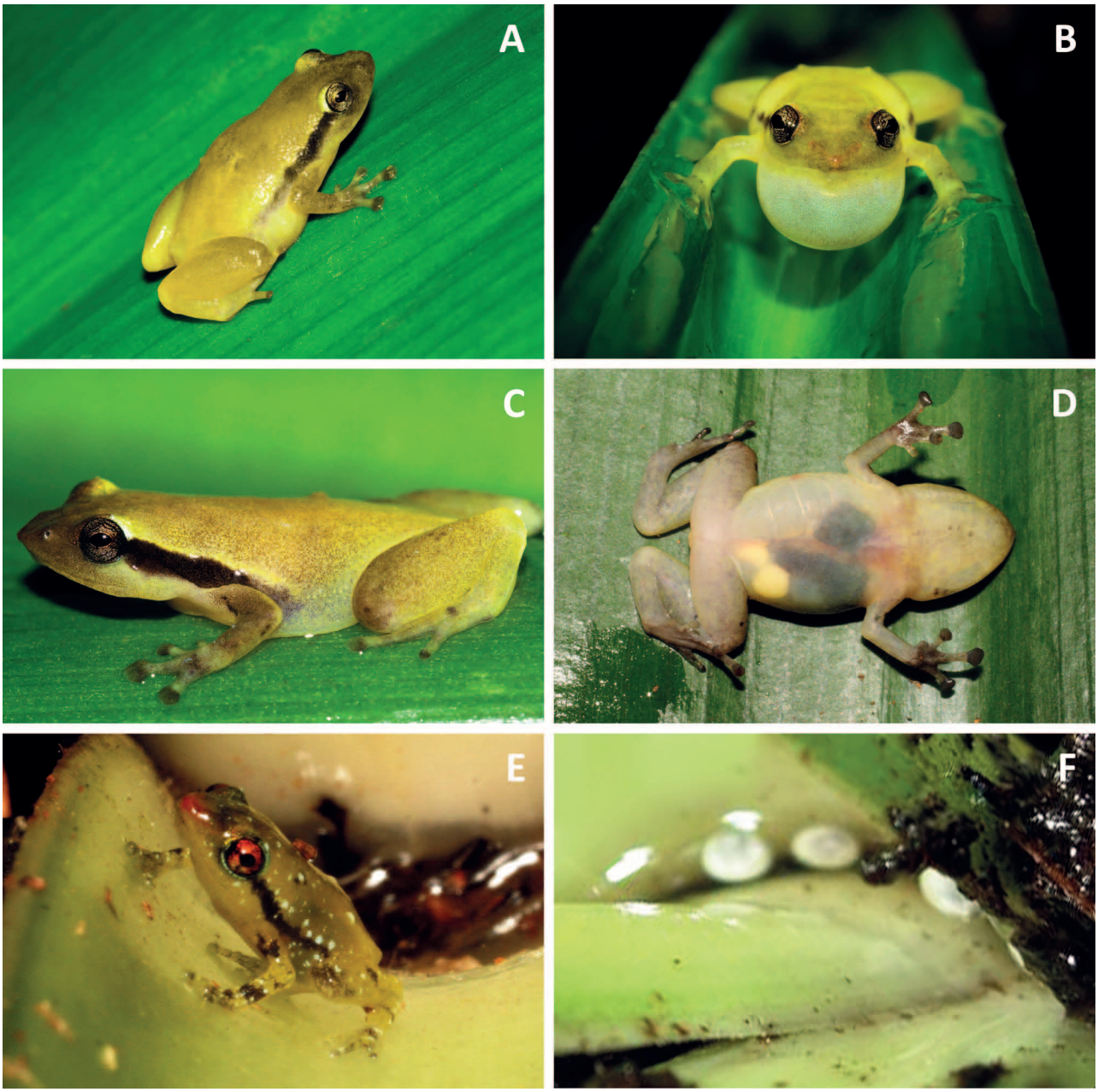

Figure 3. *Platypelis karenae* sp. n. in life: A) male holotype (MRSN A6847) in dorsolateral view; B) calling male paratype (MRSN A6369) in frontal view inflating its vocal sac; C) male paratype in lateral view (MRSN A6286); D) gravid female in ventral view with eggs in evidence (not collected); E) juvenile in frontodorsal view (not collected); (F) developing egg clutch in *Crinum* leaf axils. Photos A–C by G. M. Rosa; D–F by J. Noël.

quite variable in length in the paratypes, ranging from 2 (MRSN A5886) to about 6 mm (MRSN A6848).

Bioacoustics: Calls of *P. karenae* were recorded at Betampona (Sahambendrana campsite) on 5 November 2007, at 22:30 h, at an air temperature of 21°C; paratype MRSN A6369 (Rosa et al. 2011, track #45).

The advertisement call is composed of a short melodious note that is repeated in long series of several minutes (Fig. 5). This harmonious and whistling note has a duration of 131–145 ms (140.8 ± 2.7, n = 52). The repetition rate is 0.43/s (25.8/m). Duration of inter-note intervals is 1425–4800 ms (2284 ± 636, n = 51). The frequency is wide, ranging from 2 to 11 kHz. Four bands can be recognised: the

Figure 4. *Platypelis karenae* sp. n. natural history traits and habitats: A) live "vahona" plants (*Crinum* sp.), whose leaf axils constitute the breeding habitats of *P. karenae*; B) close-up of a leaf axil of "vahona" forming a phytotelm; C) *Pandanus* sp. (another water-retaining plant utilised by *P. karenae*); D) adult individual of *P. karenae* sheltering between two leaves of *Pandanus* sp.; E) juvenile *P. karenae* in a leaf axil of a *Crinum*; F, G) parental male guarding egg clutch in a water-filled *Crinum*. Photos A–B by G. M. Rosa; C–G by J. Noël.

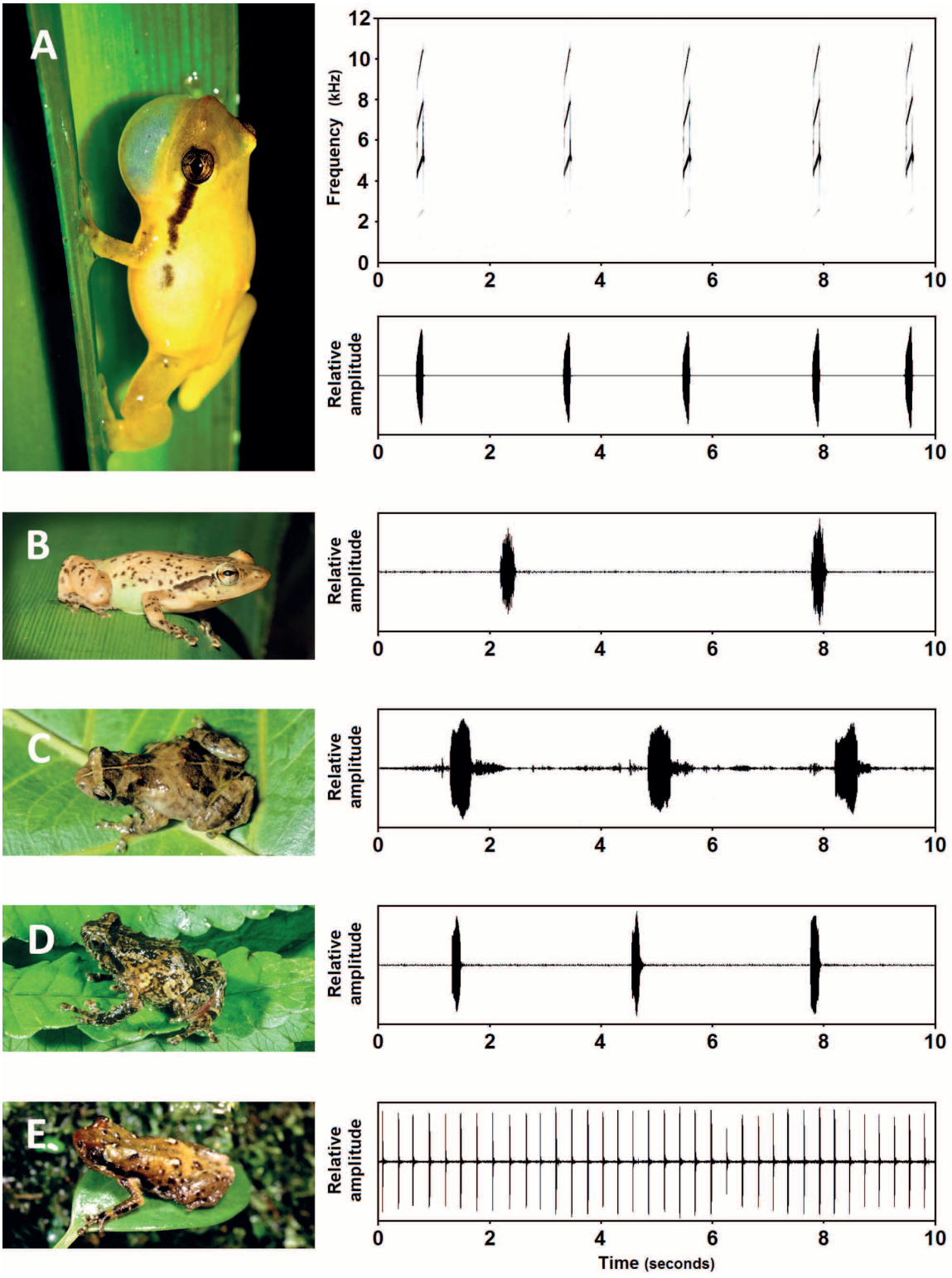

Figure 5. Comparison of habitus and advertisement call of *Platypelis* species, showing picture in life at the left and a ten seconds oscillogram section of the advertisement call at the right (plus spectrogram in *P. karenae* sp. n.): A) *P. karenae* sp. n., call recorded at Betampona (5 November 2007, 22:30, 21°C; calling male MRSN A6369); B) *P. tuberifera*, call recorded at Andasibe (12 January 1992); C) *P. ravus*, call recorded at Marojejy (15 February 2005); D) *P. barbouri*, call recorded at Andasibe (10 January 1992); E) *P. tetra*, call recorded at Anjanaharibe-Sud Special Reserve (5 February 1996). Oscillograms B–E obtained from Vences et al. (2006) tracks. Photos A–B by G. M. Rosa; C–D by F. Glaw & M. Vences; E by F. Andreone.

Table 3. Within- (bold) and among-species genetic divergence of the analysed 16S rRNA mitochondrial gene fragment, based on uncorrected pairwise p-distances for the analysed species of the genus *Platypelis*.

	P. karenae	*P. tetra*	*P.* sp. aff. *tetra* 1	*P.* sp. aff. *tetra* 2	*P. tuberifera*	*P. pollicaris*	*P. barbouri*	*P. alticola*	*P. tsaratana-naensis*	*P. milloti*	*P. grandis*
P. karenae	**0.1%**										
P. tetra	12.1%	**n/c**									
P. sp. aff. *tetra* 1	12.5%	13.0%	**0.3%**								
P. sp. aff. *tetra* 2	12.4%	12.4%	3.6%	**n/c**							
P. tuberifera	7.5%	11.2%	12.1%	11.7%	**2.0%**						
P. pollicaris	12.8%	13.8%	10.4%	10.0%	12.3%	**n/c**					
P. barbouri	11.2%	13.8%	12.0%	11.8%	12.2%	10.6%	**n/c**				
P. alticola	11.9%	12.8%	12.8%	13.2%	11.8%	12.6%	13.2%	**n/c**			
P. tsaratananaensis	12.7%	11.2%	13.6%	13.2%	12.8%	13.6%	13.0%	9.0%	**n/c**		
P. milloti	14.6%	15.0%	15.3%	15.8%	15.4%	15.8%	13.6%	16.4%	16.6%	**n/c**	
P. grandis	13.4%	12.0%	13.3%	13.4%	13.4%	12.8%	12.4%	11.6%	12.0%	16.8%	**n/c**

fundamental frequency between 2.2 and 2.8 kHz followed by a dominant band at 4.6–5.2 kHz; a third one between 7.0 and 7.8 kHz, and a higher one at 9.4–10.2 kHz. The intensity increases from the first to the second note in the beginning of the series, but remains constant at the end.

Calls of *P. karenae* are similar to those of *P. barbouri*, although this species shows a relatively lower fundamental frequency at about 4 kHz (Fig. 5D; Vences et al. 2006). The call of the sister species (*P. tuberifera*) presents a much lower note repetition rate and formant band at 23.0°C (around 2.7 kHz; Vences et al. 2006) (Fig. 5B). Calls of *Platypelis ravus* present a much longer note duration that varies between 2,504 and 3,200 ms (Fig. 5C; Vences et al. 2006), and calls of *P. tetra* differ by their substantially higher note repetition rate of 3/sec at 18.0–18.5°C, and a fundamental frequency of 3.5–4.0 kHz (Fig. 5E; Vences et al. 2006).

Natural history: The new species was always observed within plants not more than 10 m distant from streams. We found the frogs only in association with two plants: a rather small *Pandanus* sp. and a plant locally named "vahona" (Fig. 4). In general, the name "vahona" refers to plants of the genus *Aloe*, which is typical of arid areas, but at Betampona, local people use it for plants of the genus *Crinum* (Amaryllidaceae). According to C. Birkinshaw (pers. comm.), the species is *C. firmifolium*. The frogs live at the bases (axils) of the leaves of *Pandanus* and *Crinum* (Fig. 4), and will move to the leaf's periphery for vocalization. In general, males moved up and down the leaves after the sunset, with major activity being noted during rainfalls. Emissions of vocalizations were accompanied by inflating the single subgular vocal sac (Fig. 3B). When alarmed, the males would move back and hide in the leaf axil (Fig. 4D). Eggs and tadpoles were found by J. Nöel on 11 December 2010, at the campsite known as Sahambendrana, at an altitude of around 390 m. The eggs had been hidden within the leaf axils of a *Crinum* plant along a stream (Figs 3F, 4G), around 50 cm above the ground. Egg masses of *P. karenae* are rather small, containing a limited number of eggs (2–3, counted in a series of two gravid females; see Fig. 3D) of 19–24 mm in diameter. The external jelly capsule is transparent, and eggs are white (see Fig. 3F).

Distribution: This species is so far only known from the small rainforest fragment of Betampona Reserve within a narrow vertical range of 250–550 m a.s.l. on the central east coast (Fig. 1).

Molecular analyses: The mean uncorrected p-distance (for the 16S rRNA gene fragment) of *P. karenae* with *P. tetra*, *P.* sp. aff. *tetra* 1. *P.* sp. aff. *tetra* 2, and *P. tuberifera* is 12.1, 12.5, 12.4, and 7.5%, respectively (for intraspecific comparisons and comparisons with other *Platypelis* species see Tab. 3). The haplotype network reconstruction of the nuclear POMC gene (Fig. 2) shows no haplotype sharing between the analysed species of *Platypelis*, including the sympatric populations of *P. karenae* and *P. tuberifera* from Betampona.

Although *P. karenae* seems morphologically more similar to *P. tetra*, *P.* sp. aff. *tetra* 1 from Masoala and Makira, and *P.* sp. aff. *tetra* 2 from Ambodiriana, molecular data clearly suggest a more close relationship to *P. tuberifera*. Four substitutions separate the haplotypes of *P. karenae* and *P. tuberifera* at the POMC gene, and at least ten and 14 substitutions separate the haplotypes of *P. karenae* and *P.* sp. aff. *tetra* 1 and 2 (see Fig. 2 for details).

The four ML runs resulted in identical trees (not shown) with only small variation in bootstrap supports and were overall congruent with the results obtained through Bayesian analyses (Fig. 1). Our molecular analysis showed that *P. karenae* forms a robust monophyletic group (posterior probability values 1.00; ML bootstrap values = 100), and recovered a highly supported sister relationship (posterior probability values 1.00) for *P. karenae* and *P. tuberifera*. Similarly, our data clearly indicate that there is no support for the sister relationships of *P. karenae* with the other miniaturized *Platypelis* species (*P. tetra* and *P.* sp. aff. *tetra*

1 from Masoala and Makira, and *P.* sp. aff. *tetra* 2 from Ambodiriana). At the same time, they suggest a strong molecular differentiation between *P. tetra, P.* sp. aff. *tetra* 1 and *P.* sp. aff. *tetra* 2, whose phylogenetic relationships still remains unclear (see Tab. 3 for raw data of genetic differentiation and Figs 1–2). Mitochondrial data (genetic differentiation >3.5%; posterior probability values 1.00; ML bootstrap values = 100), and the absence of haplotype sharing at the nuclear POMC gene suggests that *P.* sp. aff. *tetra* 1 from Masoala and Makira, and *P.* sp. aff. *tetra* 2 from Ambodiriana may represent two undescribed species.

Conservation: *Platypelis karenae* appears restricted to Betampona, where it has been found at six sites (Sahambendrana, Sahabefoza, Maintimbato, Sahaïndrana, Vohitsivalana, and Tolongoina). The high level of training of Betampona guides (currently 6 permanent conservation agents and 8 temporary patrolling/research assistants) facilitate an impressive continued presence in the reserve, making it one of the best-managed protected areas in Madagascar. A potentially important conservation issue for Betampona is the conspicuous invasion by the introduced guava (*Psidium guajava*), which is abundant and invasive by nature, especially in the peripheral parts of the reserve and secondary growth patches of forest (Madagascar Fauna Group 2011, Rosa et al. 2012). The uncontrolled growth of the guava has the potential of replacing the typical plants of the genera *Pandanus* and *Crinum*, on which *P. karenae* lives. If this happens, the preferred habitat types and phytotelms will become rarer, together with the phytotelmic species living in them, including *P. karenae*. We therefore suggest assigning it to the category of Near Threatened (NT), because the species nearly qualifies for being listed as Vulnerable (VU) under D2: the species is confined to a single site, Betampona (2,228 ha), with a plausible threat that could impact on the species in the near future. If the threat became real, the species would be eligible for listing as Endangered, since its extent of occurrence is well within the 5,000 km² threshold under the B criterion and it would occur only in a single location (where the threat is habitat loss from agricultural activities and charcoal production) and there would be a continuing decline in the quality and area of habitat, qualifying the species for the criteria B1ab(iii).

Discussion

The description of a new small *Platypelis* from Betampona rainforest indicates that the taxonomic discovery curve of Malagasy frogs is still far from reaching saturation point, even in regions that are relatively easy to access. Perl et al. (2014) have predicted that the final number of frog species will be in excess of 500 species for Madagascar, and the current trend of new species description appears to confirm this notion (e.g., Andreone et al. 2010, Glaw et al. 2010, Vences et al. 2010, Crottini et al. 2011, Köhler et al. 2011, Rakotoarison et al. 2012, Klages et al. 2013, Penny et al. 2014). Amongst the wealth of Malagasy frogs, cophyline microhylids still represent one of the most enigmatic groups, and many cryptic species, possibly micro-endemic, are still awaiting description. However, it is surprising to note that recent survey work at Betampona has revealed about 21 amphibian taxa, corresponding to 1/3 of the species found there, that have not yet been described formally (Rosa et al. 2012). This high number of candidate species (among which are five undescribed species of the genus *Platypelis*) is possibly unique in eastern Madagascar and likely due to Betampona representing the last major fragment of low-altitude rainforest along the east coast of Madagascar.

Due to the problem of morphological identification of cryptic species we believe that other species of small phytotelmic *Platypelis* may be in need of further investigation, too. In fact, we found similar individuals at Makira and in the Maroantsetra area (here named *P.* sp. aff. *tetra* 1) and *P.* sp. aff. *tetra* 2 at Ambodiriana. All these small *Platypelis* species (*P. karenae*, *P. tetra*, *P.* sp. aff. *tetra* 1 and 2) and the larger *P. tuberifera* share the common ecological trait of depending on *Pandanus* phytotelms. Several of these smaller *Platypelis* live in the more northern region of Madagascar that also appears to constitute a centre of differentiation of their genus, with 11 species (of the 14 species treated by Glaw & Vences 2007) being known only from there (Wollenberg et al. 2008).

We foresee that other forest blocks along the eastern and northeastern coast of the island will host further undescribed *Platypelis* species. For these reasons, conservation action is needed to protect representative lowland forest at as many sites as possible, and an in-depth phylogenetic analysis aiming at resolving the still-unclear phylogenetic relationships within this genus is required. The discovery of many new species in Betampona and the evident biodiversity richness in residual fragments of rainforests demonstrate how species conservation, especially for amphibians, is often closely related to the conservation of even modestly sized relict forests.

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