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Gray versus yellow ventral coloration: Identity, distribution, color polymorphism and molecular relationships of the microhylid frog *Platypelis mavomavo* Andreone, Fenolio & Walvoord, 2003

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Abstract

The Malagasy frog *Platypelis mavomavo* from Ambolokopatrika in the North East of Madagascar was originally diagnosed based on its bright yellow venter, but only limited information on this species has become available after its initial description in 2003. Several *Platypelis* specimens with yellow ventral color have been erroneously assigned to this species due to a lack of DNA sequences from the *P. mavomavo* type series. On the other hand, the candidate species *Platypelis* sp. Ca10 from Andranomapanga in the Northern Central East of Madagascar with gray ventral color has been defined based on its genetic differentiation from other nominal *Platypelis* species. Here we study the genetic variation of *P. mavomavo* and *P.* sp. Ca10 based on mitochondrial (16S rRNA) and nuclear-encoded (RAG-1) genes, including a newly determined sequence from the *P. mavomavo* holotype, which was studied using a museomics approach. We find only limited genetic variation among the samples studied, and this variation is unlinked to ventral coloration but instead reflects geographic distribution. We, therefore, conclude that *P.* sp. Ca10 is a gray-colored variant of *P. mavomavo,* and that *P. mavomavo* is rather widespread in the North East and Northern Central East of Madagascar, with populations in areas bordering the North West (Ambohitantely) and Sambirano (Ampotsidy) geographic regions, and the yellow-bellied morph restricted to the North East (Makira, Ambolokopatrika). Due to the range extension of *P. mavomavo*, the conservation status of the species requires re-assessment.

Key words: Amphibia; Anura; ventral coloration; Cophylinae; molecular genetics; museomics; color polymorphism.

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Introduction

According to Andreone *et al*. (2003), *Platypelis mavomavo* (Microhylidae, Cophylinae), is a medium sized (24–29 mm) arboreal frog with a uniformly yellow ventral side. The species was discovered in 1997 at Ambolokopatrika in northeastern Madagascar during a rainy night climbing above the ground on small tree trunks. It was diagnosed to have vestigial webbing between toes, a smooth to warty dorsal surface, and a yellow venter; its call is still unknown.

In the past two decades, several other specimens presenting a fully or partially yellowish ventral color were assigned to this species, although their molecular relationships are still unclear. Indeed, Andreone *et al*. (2005), while presenting the first comprehensive molecular phylogeny of Cophylinae, mentioned that their analyses include one individual of *P.* cf. *mavomavo*, which was reported as "*Platypelis* sp. 3" in the species list of the article (voucher specimen MRSN A2630 from Tsaratanana). A further molecular phylogenetic study of cophyline frogs (Wollenberg *et al*. 2008) did not mention *P*. *mavomavo* but included sequences of this same individual specimen (MRSN A2630) as "*P.* sp. 3". Glaw and Vences (2007), in addition to the real *P*. *mavomavo,* mentioned two candidate species that have affinity with *P*. *mavomavo*: "*P.* sp. aff. *mavomavo* 1" which corresponds to *P.* sp. 3 from Tsaratanana as previously reported by Andreone *et al*. (2005) and Wollenberg *et al*. (2008), and "*P.* sp. aff. *mavomav*o 2", which was later formally described by Glaw *et al*. (2012) as *P. ravus*.

Vieites *et al*. (2009) generated the first 16S rRNA gene sequence of the real *P. mavomavo*, obtained from the paratype MRSN A2434 (now ZSM 41/2011), and used it in their phylogenetic tree, but erroneously quoted the GenBank accession number FJ559285 which instead corresponds to "*P*. sp. 3" from Tsaratanana. In their effort to provide a standardized numbering system for the candidate species of Malagasy frogs, Vieites *et al*. (2009) renamed the candidate species "*P*. sp. 3" of Andreone *et al*. (2005) and Wollenberg *et al*. (2008) to "*P*. sp. 8". These authors also defined a further candidate species, *P*. sp. 10 (based on the 16S sequence of the voucher specimen ZCMV 8866 from Andranomapanga; GenBank accession number: FJ559292), which was placed sister to the real *P*. *mavomavo* (paratype MRSN A2434). Individuals of this candidate species, however, have a gray (rather than yellow) ventral color (Glaw & Vences 2007). The same specimen of *P*. sp. 10 (ZCMV 8866) has been recorded as both *P. mavomavo* for cytochrome-oxidase 1 (COI) (Perl et *al. 2*014) and as P. *s*p. 10 for 16S rRNA (Vieites et al. 2009), highlighting the confusion over this species.

Rakotoarison *et al*. (2012) included the same 16S sequence of *P.* sp. 8 (sensu Vieites et al., 2009) from Tsaratanana in their tree, but without any further information on this candidate species. Perl *et al*. (2014) provided sequences of the mitochondrial DNA barcoding gene COI for the two candidate species, referring to them as *P.* sp. Ca8 (for *P.* sp. 8 sensu Vieites *et al*., 2009), and as "*P*. *mavomavo* Andranomapanga" (for *P.* sp. 10 sensu Vieites et al., 2009 – corresponding to *P.* sp. Ca10 as used herein), anticipating genetic similarities of this frog to *P. mavomavo.* Finally, Scherz *et al*. (2016) used the same naming scheme as Perl *et al*. (2014).

To solve the taxonomic conundrum of these frogs, we here present novel molecular and morphological data for previously studied as well as newly collected specimens. We also managed to obtain a 16S sequence from the holotype of *P. mavomavo* (MRSN A2435) using a museomic approach, and thereby are able to conclusively assign this scientific name to a cluster containing frogs with gray as well as yellow venter.

Materials and Methods

This study drew on specimens of *Platypelis* collected during several different field campaigns. Animals were localized both during the day by inspecting tree holes, and by opportunistic searches with headlamps at night. Specimens were euthanized in MS-222 solution, fixed in 90% ethanol and preserved in 70% ethanol. In other cases, buccal swabs were taken and preserved in 99% ethanol, and specimens released at the site of capture. Vouchers were deposited in either the Zoologische Staatssammlung München (ZSM) or in the amphibian collection of the Mention Zoologie et Biodiversité Animale of the University of Antananarivo (UADBA-A). Field numbers beginning with FGZC are from Frank Glaw, FGMV are from Frank Glaw and Miguel Vences, KAMUT and KAMUS are from Katherine E. Mullin, MVTIS and ZCMV are from Miguel Vences, and MSZC are from Mark D. Scherz. Additional samples are from the Museo Regionale di Scienze Naturali of Torino, Italy (MRSN). Measurements were taken to the nearest 0.1 mm by various researchers using digital calipers. For this study, five specimens were newly

measured (Table 1). The measurement scheme largely follows that used by Rakotoarison *et al*. (2012): we measured snout–vent length (SVL), maximum head width (HW), tibia length (TIBL), hindlimb length (HIL), head length (HL), horizontal eye diameter (ED), eye–nostril distance (END), nostril–snout tip distance (NSD), nostril–nostril distance (NND), horizontal tympanum diameter (TD), hand length (HAL), foot length (FOL), foot length including tarsus (FOTL), and forelimb length (FORL). Measurements are compared with those taken by Franco Andreone and published in Andreone *et al.* (2003); equivalency was compared by examining ratios to SVL, and checking for systematic differences by measurer. No differences were identified, but we did note that Andreone *et al.* (2003) reported TIBL measurements identical to their SVL measurements, which must have been an error; we give these as NAs in Table 1. Statistical comparison of measurements (t-tests) was carried out in Microsoft Excel, with all measurements size-corrected except SVL itself, by dividing by SVL. T-tests were carried out with two-tails and assuming equal variance. 11 comparisons were made, and the Bonferroni correction set the α to 0.00455 (=0.05/11). Males and females were not analyzed separately due to our small sample size.

TABLE 1. Morphometric measurements (all in mm) of specimens herein assigned to *Platypelis mavomavo.* Individuals from the MRSN collection represent the original type series of the species (MRSN A2435, in bold, being the holotype, the other individuals paratypes). Measurements of MRSN specimens were taken by F. Andreone and are extracted from the original description (Andreone *et al.* 2003); ZSM specimens (except ZSM 41/2011) were newly measured by MV. Note that Andreone *et al.* (2003) reported TIBL measurements identical to their SVL measurements, which must have been an error; we give these as NAs.

Museum number	Field number	locality	Sex	SVL	HW	HL	ED	TD	END	NSD	NND	HAL	FORL	HIL	FOTL	FOL	TIBL
MRSN A2435	FN 7177	Ambolokopatrika	M	26.5	9.3	9.7	4.1	1.7	2.5	2.1	3.0	8.1	18.8	NA	NA	11.7	NA
MRSN A2432	FN 6799	Ambolokopatrika	M	28.6	11.3	9.6	3.6	1.7	2.5	2.4	2.7	8.6	20.7	NA	NA	12.8	NA
ZSM 41/2011	FN 7239	Ambolokopatrika	M	24.4	8.4	8.5	2.9	2.1	2.5	2.1	2.6	8.8	17.5	NA	NA	10.7	NA
MRSN A2431	FN 6693	Ambolokopatrika	F	27.9	9.9	9.7	4.0	1.8	2.4	2.6	3.2	8.5	18.3	NA	NA	12.8	NA
MRSN A2433	FN 7398	Ambolokopatrika	F	26.8	9.4	8.8	3.4	1.6	2.0	2.4	3.6	8.1	19.4	NA	NA	11.7	NA
ZSM 162/2022	FGZC 6553	Makira	M	27.8	10.1	9.9	3.6	2.4	1.9	2.0	1.8	9.8	18.4	42.4	20.1	13.4	12.8
ZSM 1755/2008	ZCMV 8866	Andranomapanga	?	25.6	9.0	10.0	3.3	2.0	2.0	2.2	2.6	7.3	16.0	31.4	15.2	10.0	9.7
ZSM 109/2016	MSZC 16	Ampotsidy	M?	21.8	7.8	7.7	3.0	1.8	1.3	1.4	2.3	6.8	9.3	29.2	13.5	8.9	8.7
ZSM 108/2016	MSZC 4	Ampotsidy	F	32.3	11.1	10.4	3.7	2.2	2.3	1.8	2.4	9.2	19.9	41.6	20.4	13.6	11.8
ZSM 110/2016	MSZC 108	Ampotsidy	M?	23.5	8.3	7.6	3.1	1.7	1.8	1.4	2.7	6.9	14.8	31.9	14.5	9.6	9.4

For molecular analysis, we complemented existing data for two adjacent fragments of the mitochondrial 16S rRNA gene (here called 16S-5' and 16S-3' to refer to their position close to the 5' or 3' terminus of the gene) from previous studies (e.g., Andreone *et al*. 2005; Wollenberg *et al*. 2008; Rakotoarison *et al*. 2012, 2015, 2019a, 2020; Rosa *et al*. 2012, 2014; Scherz *et al*. 2019; Glaw *et al*. 2020). After compiling all sequences available from GenBank for species of *Cophyla* and *Platypelis*, we selected a subset of sequences based on the following criteria: (1) preferably, we selected individual samples for which both fragments were available; (2) per species, up to three samples were selected covering different sites across their distribution ranges; (3) from species with colorful (red or yellow) ventral pattern, up to five samples were included; (4) of the focal lineages (*P. mavomavo* and *P.* sp. Ca10) all available samples were included; (5) only for one taxon (*C. occultans*) a chimeric sequence was included, i.e., 16S-5' and 16S-3' fragments from different individuals were combined.

For new samples analyzed in the present study, we used muscle tissue samples taken in the field from animals after euthanasia but before fixation and preserved in 99% ethanol, or buccal swabs (KAMUS numbers). The samples were processed in various laboratories (Universities of Braunschweig or Cardiff) using standard methods, as explained in the following. Genomic DNA was extracted using routine protocols (either a Qiagen

DNeasy blood and tissue kit, or a salt extraction protocol: Bruford *et al.* 1992) and the two 16S fragments were PCR-amplified with primers 16SL3 (AGCAAAGAHYWWACCTCGTACCTTTTGCAT) and 16SAH (ATGTTTTTGATAAACAGGCG) of Vences *et al*. (2003) for 16S-5', and 16Sar-L (CGCCTGTTTATCAAAAACAT) and 16SBr-H (CCGGTCTGAACTCAGATCACGT) of Palumbi *et al*. (1991) for 16S-3', with PCR protocols as given by Vences *et al.* (2003). For 16S-5' this was: 90 s at 94 °C, followed by 33 cycles of 45 s at 94 °C, 45 s at 52 °C, 90 s at 72 °C, and a final extension step of 300 s at 72 °C; and for 16S-3': 90 s at 94 °C, followed by 33 cycles of 45 s at 94 °C, 45 s at 55 °C, 90 s at 72 °C, and a final extension step of 300 s at 72 °C. We furthermore amplified a fragment of the nuclear recombination-activating gene 1 (RAG-1) with primers Rag1_Coph_F1 (CGTGATCGGGTAAAAGGTGT) and Rag1_Coph_R1 (TCGATGATCTCTGGAACGTG) of Rakotoarison *et al*. (2015), with the following PCR protocol: 120 s at 94 °C followed by 35 cycles of 20 s at 94 °C, 50 s at 53 °C, 180 s at 72 °C, and a final extension step of 600 s at 72°C. Purified PCR products were sequenced on capillary sequencers using the respective forward primers, chromatograms were checked by eye, and poor-quality terminal sections were trimmed with CodonCode Aligner 3.7.1 (Codon Code Corporation, Dedham, MA, USA). Newly obtained sequences were submitted to GenBank (accession numbers OR427372–OR427398, OR501884–OR501893, and OR461473). Overall, a total of 33 sequence fragments of 27 specimens of *Platypelis* and *Cophyla* were newly obtained for this study ; a table with all sequences, accession numbers, voucher numbers and localities has been archived in the Zenodo repository (DOI: 10.5281/zenodo.8229715), along with alignments and tree files.

The holotype of *P. mavomavo* (MRSN A2435), housed at the Museo Regionale di Scienze Naturali of Torino, was loaned to the University of Potsdam, Germany for the purpose of this study. Tissue was taken from the liver in a minimally-destructive manner using sterile scalpels and tweezers, through a pre-existing cut in the flank. Prior to extraction, the sample was weighed and incubated in a Guanidine Thiocyanate (GuSCN) based extraction buffer solution at 37°C overnight. The next day, a total volume of 25 µl genomic DNA was extracted following the protocol of Rohland *et al*. (2004), including several consecutive steps as described in Straube *et al*. (2021). The yield of DNA was quantified based on 1 µl DNA extract using the Qubit dsDNA HS Assay Kit 0.2–100 ng/µl (Life Technologies, Carlsbad, California, US) according to the instructions of the manufacturer. Subsequently, a maximum of 13 ng DNA was used as input for single-stranded library preparation following the protocol of Gansauge *et al*. (2017). All lab work prior to qPCR was conducted in a dedicated DNA facility at the University of Potsdam, Germany, which meets all requirements to work with historical samples (see Fulton & Shapiro 2019) and extraction and library blanks were run alongside all samples to check for contamination. Final library concentrations and fragment length distributions were assessed using a 2200 TapeStation (Aligent Technologies) assay. Sequence data was obtained through shotgun-sequencing of approximately one million 75 bp single-end reads using an Illumina Nextseq 500/550 sequencing platform at the University of Potsdam, following the procedure described in Paijmans *et al*. (2017). The quality of the obtained reads was visualized twice using FastQC (https://www.bioinformatics.babraham.ac.uk), both before and after trimming of Illumina adapter sequences and discarding reads shorter than 30 bp with cutadapt v1.12 (Martin 2011). Mapping of reads was performed in Geneious Prime v.2022.0.1 (Biomatters Ltd., Auckland, New Zealand) using a consensus sequence of 16S for the genus as a reference, which was derived from an alignment including sequences from all currently recognized *Platypelis* species that were publicly available on GenBank. Individual mapping was run for 100 iterations using a mapping quality filter of 30 and medium-low sensitivity options. The obtained contigs were assembled into a consensus sequence of 16S with missing sections in-between contigs coded by the letter "N".

All sequences (those from previous studies and newly determined) were combined with their metadata (voucher numbers, geographical provenance and GenBank accession numbers) in a spreadsheet (available along with analysis files in the Zenodo repository under DOI 10.5281/zenodo.8229715). We then used Concatenator (Vences *et al*. 2022) which is part of the iTaxoTools package (Vences *et al*. 2021) to concatenate and align the two 16S fragments. Alignment was carried out in Concatenator using the G-INS-I algorithm of MAFFT (Katoh & Standley 2013). We used MEGA7 (Kumar *et al*. 2016) to select the best-fitting substitution model (GTR+G) for the concatenated data based on the Bayesian Information Criterion and ran a phylogenetic analysis under the Maximum Likelihood (ML) optimality criterion in RAxML (Stamatakis 2014) as implemented in raxmlgui (Edler *et al*. 2021), with 500 thorough bootstrap replicates to assess node support. Based on previous multi-gene analyses (Scherz *et al*. 2016) we used *Cophyla* as outgroup and rooted the tree at the *Cophyla/Platypelis* node.

We analyzed the sequences of the nuclear-encoded RAG-1 gene separately from the mitochondrial gene, in order to understand if the mitochondrial relationships suggested by our analysis may be influenced by introgression.

To graphically represent the relationship among alleles (haplotypes) of the RAG-1 gene fragment, we used a network approach which is more straightforward than a tree to visualize identical haplotypes shared among subsets of individuals (in this case, yellow- vs. gray bellied *Platypelis* initially assigned to *P. mavomavo* vs. *P.* sp. Ca10). Haplotypes were inferred with the PHASE algorithm (Stephens *et al*. 2001) implemented in the DnaSP software (Version 5.10.3; Librado & Rozas 2009), and an ML tree was inferred under the Jukes-Cantor substitution model in MEGA7 (choosing this simple model to avoid overparameterization, in order to weigh each mutation equally in this dataset that contained a maximum difference of five mutations over the entire alignment length). Then this tree, together with the respective alignment, was used as input for Haploviewer (written by G. B. Ewing; http://www.cibiv.at/~greg/haploviewer), a software that implements the methodological approach of Salzburger *et al*. (2011).

To assess genetic divergences, we calculated uncorrected pairwise divergences of the MAFFT-aligned sequences with MEGA7. For this calculation, we used only the 16S-3' fragment which has been more commonly used in previous molecular taxonomic assessments and genetic distance comparisons in Malagasy frogs (e.g., Vieites *et al*. 2009); and we used a reduced dataset with only 12 sequences of the focal taxa with complete sequences of this 16S fragment over an alignment length of 498 bp (with only one nucleotide missing in one of the sequences), plus some information of the *P. mavomavo* holotype and paratype (with only a short part of the fragment of 185–191 bp available). The alignment file is available from the Zenodo archive, DOI: 10.5281/zenodo.8229715.

Results

Molecular relationships

The alignment of the two concatenated 16S fragments had a length of 1229 bp for a total of 71 included samples. The resulting ML mitochondrial gene tree (Fig. 1A) generally agrees with previous phylogenies of the *Cophyla+Platypelis* clade and we here highlight only the position of the focal taxa. All samples of *P. mavomavo* and *P.* sp. Ca10 together are recovered as a highly supported monophyletic group (Bootstrap Support, BS = 100%), and this group is placed together with *P. alticola* and *P.* sp. Ca8 in a poorly supported clade (BS = 56%). Within the *P. mavomavo*/Ca10 group, sequences cluster according to locality (compare with map in Fig. 2) and not according to ventral color: gray-venter samples from Ampotsidy cluster with each other (BS = 95%) and are placed in a clade with yellow-venter samples from Makira and the type locality of *P. mavomavo*, Ambolokopatrika (BS=65%); the yellow-venter samples are arranged paraphyletically in the tree and do not form a monophyletic group. The sample from Andranomapanga is placed in a clade with samples from the geographically neighboring site, Ambohitantely (BS = 65%).

Genetic distances in the 16S-3' fragment amount to 5.4–6.3% between *P.* sp. Ca8 and the *P. mavomavo*/Ca10 clade. Within the *P. mavomavo*/Ca10 clade, distances amounted up to 2.6–3.0% between the gray-venter samples from Ampotsidy and other gray-venter samples (from Ambohitantely, Andranomapanga, Anjozorobe, and Ambatovy). The yellow-venter sample from Makira had differences of 2.2% to samples from Ampotsidy, and 0.8–1.4% to gray-venter samples from other sites. For the holotype and paratype of *P. mavomavo* (MRSN A2435 and ZSM 41/2011 [formerly MRSN A2434], respectively) from Ambolokopatrika, both with yellow venter according to the original description, only short sequences of 189 and 193 bp were available. These sequences were missing several of the hypervariable (loop) regions of the 16S fragments and therefore had overall lower distances to other samples: no substitution was observed between them (0% distance) and they differed by 1.0–1.1% from the yellow-venter specimen from Makira, and by 0.6–1.6% from the remaining (gray-venter) samples.

The haplotype network based on RAG-1 alleles (386 bp) of 10 samples obtained via phasing agreed with the mitochondrial tree in grouping samples independently of their ventral color (see Fig. 1A and Fig. 1B). The two alleles of the sole yellow-venter specimen for which a RAG-1 sequence could be obtained (from Makira) were identical with each other and with those of a specimen from Ampotsidy, in agreement with the clustering of the Makira and Ampotsidy samples in the mitochondrial tree. Other samples from Ampotsidy were represented by a second haplotype differing by a single mutation, while the two included samples from Anjozorobe and Andranomapanga differed by the samples from Ampotsidy by two or three mutational steps, respectively.

Morphology and coloration

Specimens assigned to the *P. mavomavo*/Ca10 clade by DNA sequences were morphologically rather uniform except for a bright yellow venter in living individuals from Ambolokopatrika (type series) and Makira, and a

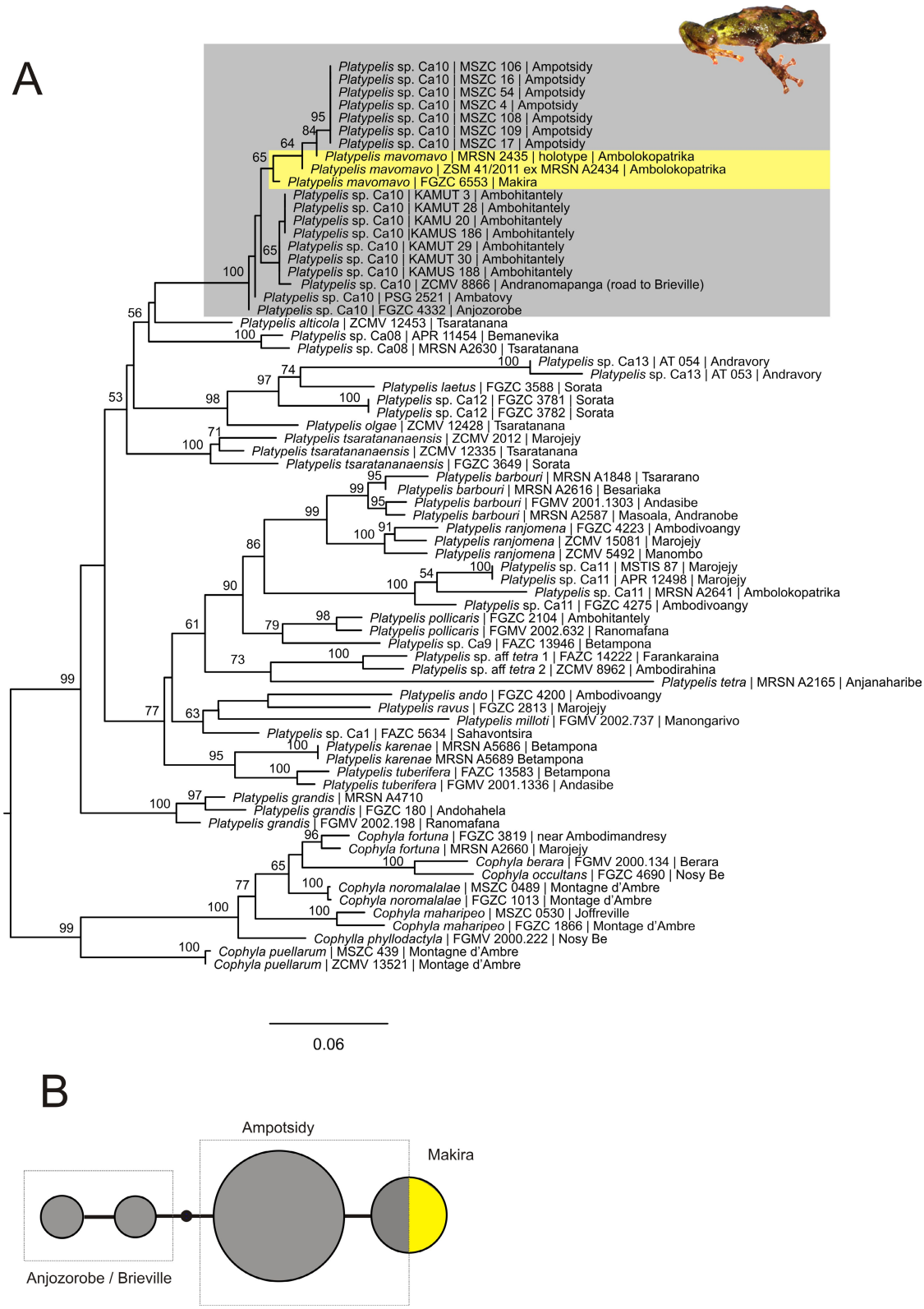

FIGURE 1. Molecular differentiation and relationships of specimens of *Platypelis mavomavo*. (A) Maximum Likelihood tree calculated from a 1229 bp alignment of two concatenated fragments of the mitochondrial 16S rRNA gene for selected specimens of *Cophyla* and *Platypelis*. Numbers at nodes are bootstrap proportions in percent (only shown if >50%). The focal *P. mavomavo*/Ca10 clade is color-highlighted depending on the yellow vs. gray ventral color of the respective specimens. (B) Haplotype network reconstructed from 10 phased sequences of the nuclear-encoded RAG-1 gene (386 bp) in samples assigned to *Platypelis mavomavo* and *P.* sp. Ca10. Haplotypes are colored depending on the yellow vs. gray ventral color of the respective specimens. Boxes indicate haplotypes from specific areas.

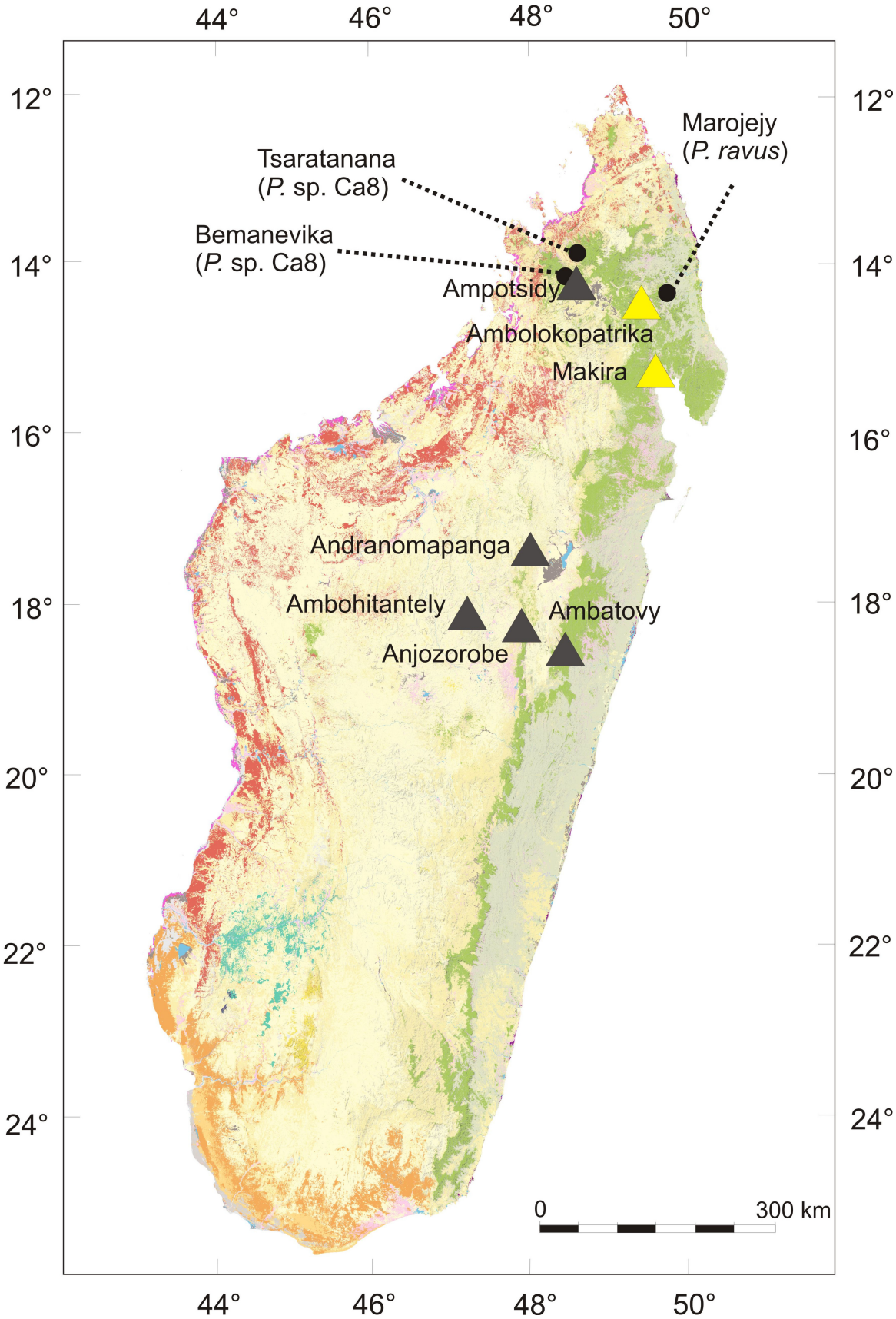

FIGURE 2. Distribution map showing confirmed records of *Platypelis mavomavo* as defined herein. Yellow and gray symbols mark localities where specimens are characterized by a yellow or gray ventral color. Round black dots indicate other localities discussed in the text where other *Platypelis* species or candidate species with fully or partially yellow venter occur (*P. ravus, P.* sp. Ca8).

uniformly gray venter in the remaining individuals (Fig. 3–4). In the newly collected Makira specimen, the yellow color was completely faded upon examination about one year after preservation; also the holotype has no trace of yellow left on the ventral side (Fig. 5). Dorsally, the ventrally yellow specimens in life had a light brownish color with large symmetrical dark brown markings (Fig. 4). In the ventrally gray specimens, dorsal ground color had more contrasted patches ranging from beige to light brown or greenish, but also with a distinct and symmetrical dark pattern (Fig. 3).

Five frogs were newly measured; details are given in Table 1. The ventrally yellow specimens from Ambolokopatrika and Makira had snout–vent lengths of 24–28 mm in males and 27–28 mm in females, while the ventrally gray specimens from Ampotsidy had 22–24 mm in probable males and 32 mm in a confirmed female (Table 1). Field measurements of the ventrally gray specimens from Ambohitantely revealed SVLs of 34–35 mm in probable females, and 20–32 in additional unsexed specimens. Across all measurements, there were no statistically significant differences after size and Bonferroni correction, except relative foot length (FOL): 0.436–0.482 in yellow-bellied specimens, 0.391–0.421 in gray-bellied specimens (two-tailed t-test, P = 0.002351472, Bonferroni $\alpha = 0.00455$). However, we cannot exclude that these differences are the result of different researchers taking the measurements for specimens from Ambolokopatrika.

Note that some details of head shape and a vertical pupil shape were wrongly shown for the holotype of *P. mavomavo* in a drawing of Andreone *et al.* (2003) and subsequently corrected in Andreone *et al.* (2004). We have here identified a further error, i.e. the incorrect measurements given for TIBL.

Distribution and natural history

According to the genetic data presented herein (Fig. 1) *P. mavomavo* occurs at the following localities (Fig. 2): (1) Ambolokopatrika (MRSN A2433 and MRSN A2435 from the type locality Antsinjorano, 14.5433°S, 49.4300°E, 975 m a.s.l.; MRSN A2431 from Andemakatsara, 14.5300°S, 49.4417°E, 875 m; MRSN A2432 from Andranomadio, 14.5400°S, 49.4383°E, 890 m a.s.l.); (2) Ambatovy (MVTIS 29308, ca 18.83°S, 48.31°E, ca 1000 m a.s.l.); (3) Ambohitantely (KAMUT 3, 20, 28–30, KAMUS 186, 188, ca 18.20°S, 47.29°E, 1465–1556 m a.s.l.); (4) Ampotsidy (ZSM 108–110/2016 = MSZC 4, MSZC 16, MSZC 108, ca 14.41689°S, 48.71435°E, 1244–1447 m a.s.l.); (5) Andranomapanga (ZSM 1755/2008 = ZCMV 8866, 17.7052°S, 47.9310°E, 1086 m a.s.l.); (6) Anjozorobe (ZSM 389/2010 = FGZC 4332, 18.46294°S, 47.93812°E, 1287 m a.s.l.); (7) Makira (ZSM 162/2022 = FGZC 6553, 15.17806°S, 49.62444°E, 891 m a.s.l.). These localities are located between 875–1556 m a.s.l.

According to Andreone *et al.* (2003), the species was first discovered at the western slope of Anjanaharibe-Sud in 1996, but the specimen was only photographed and not captured, and this locality is therefore not genetically confirmed so far.

In Ambohitantely Special Reserve seven frogs were encountered, all in one of the northern forest fragments in April and May 2019. All specimens were encountered in tree holes during morning surveys (08:00–09:20). One individual was found in a dry tree hole (1 m from the ground) in slope habitat >10 m from a water source at 1556 m a.s.l. This individual was a dorsally mottled mossy green, black and brown color. The other six individuals were found in two tree holes at 1465 m a.s.l in riparian habitat, three individuals sharing each hole. One water-filled tree hole was 1 m from the ground overhanging the stream, while the other was 1 m from the same stream 1.5 m from the ground. These six individuals were cream/mint green/brown in dorsal color. Three of the individuals were ovigerous, suggesting that this species breeds late in the wet season in water filled tree holes. No advertisement calls were recorded. No individuals were recorded in the core forest in the reserve, in the other four forest fragments surveyed or Ankafobe reserve, across 603 survey hours, suggesting this species either has cryptic habitats, or is indeed rare at this locality.

In Ampotsidy (Bealanana District), *P. mavomavo* individuals were collected in large to small fragments of primary rainforest in December 2015, from 1244–1447 m a.s.l. Some individuals were found under tree bark during the day, but most were encountered active on tree trunks or vines at night. No calls of any *Platypelis* were heard in Ampotsidy between December 2014 and January 2015, despite the presence of both this species and *P.* cf. *grandis*. MSZC 0016 was an ovigerous female with eggs visible through the venter, suggesting that the species was nonetheless in breeding condition at this time of year. Further efforts are needed in the diminutive and diminishing forest fragments around the Bealanana District to establish to what extent this species is present in them. It is noteworthy however that studies in Bemanevika (Rabearivony *et al.* 2010) have so far not found records of this species, despite the proximity of that area to Ampotsidy.

The specimen from Makira (collected on 16 April 2022) was an adult, ovigerous female with externally visible eggs in the body cavity.

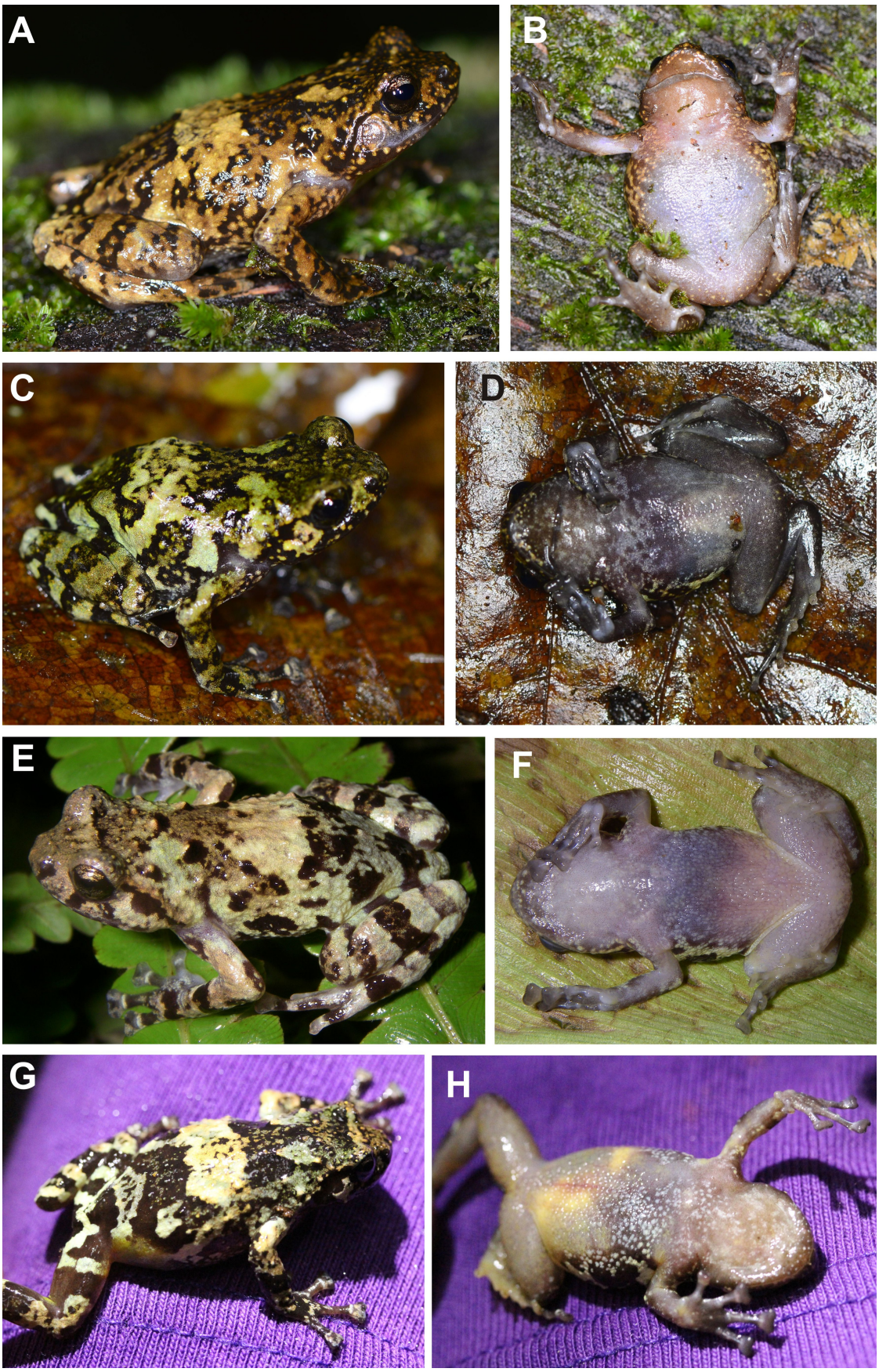

FIGURE 3. *Platypelis mavomavo* (specimens previously named *P*. sp. Ca10) with grayish colored ventral side in life, in dorsolateral and ventral views. (A–B) ZSM 108/2016 (MSZC 4) from Ampotsidy; (C–D) ZSM 109/2016 (MSZC 16) from Ampotsidy; (E–F) ZSM 1755/2008 (ZCMV 8866) from Andranomapanga; (G–H) specimens from Ambohitantely.

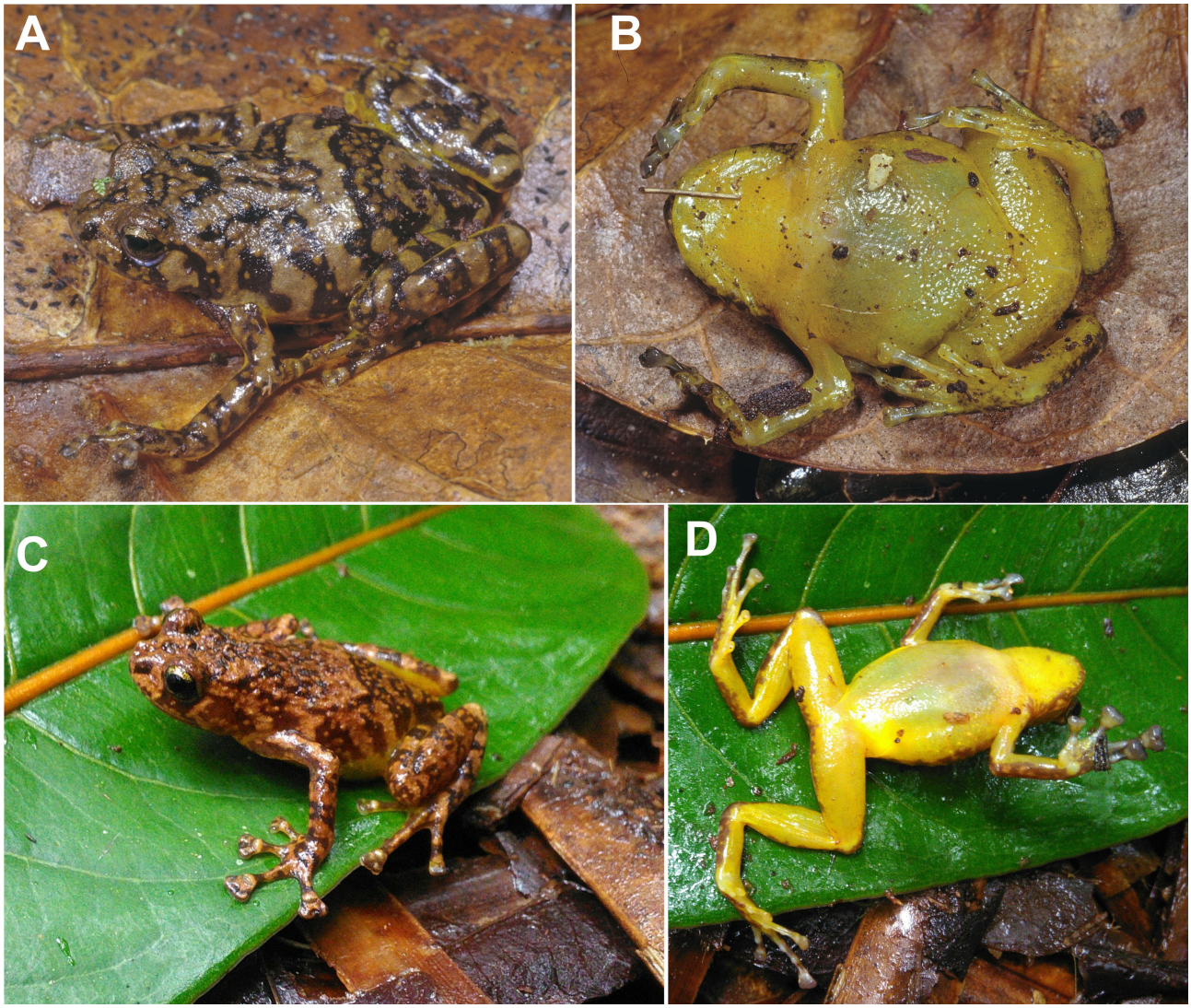

FIGURE 4. *Platypelis mavomavo* with yellowish colored ventral side in life, in dorsolateral and ventral views. (A–B) holotype MRSN A2435 from Ambolokopatrika; (C–D) ZSM 162/2022 (FGZC 6553) from Makira.

Discussion

This study clarifies the relationship of *Platypelis* specimens previously assigned to *P. mavomavo* and *P.* sp. Ca10, and provides novel information on their geographical distribution and natural history. Molecular data presented herein clearly suggest the existence of a single evolutionary lineage of *Platypelis* frogs comprising individuals with yellow ventral color (distributed in the North East of Madagascar) and gray ventral color (in the Northern Central East, including areas bordering the North West and Sambirano regions). The intertwined position of the yellow-bellied individuals inside the phylogroups of gray-bellied specimens, along with the encountered haplotype sharing of these frogs in the nuclear marker analyzed, makes a two-species scenario extremely unlikely. Instead, it seems that the yellow-bellied individuals are from lower elevations (875–975 m a.s.l.) than the gray-bellied populations (ca. 1000–1556 m a.s.l.). Despite some detailed patterns and a substantial intra-populational variation, there is a general similarity in dorsal pattern among all specimens examined, and we could not identify major morphological differences between yellow- and gray-bellied specimens. Furthermore, because our data set includes sequences of the holotype and of one paratype of *P. mavomavo*, we can confidently assign the nomen to this genetic lineage. However, it is important to highlight that due to an incomplete set of sequences (different non-overlapping fragments of 16S for some of the specimens in our analysis), phylogeographic relationships among populations of *P. mavomavo* remain in need of further study. This is especially true because the sequences that could be recovered

FIGURE 5. Preserved holotype of *Platypelis mavomavo* MRSN A2435 in (A) dorsal and (B) ventral views. Not to scale (see Table 1 for original measurements).

from the liquid-preserved holotype and paratype were too short to robustly solve their relationships with the other specimens.

For several years, it has been known that many species of widespread frogs in Madagascar, are complexes of cryptic species, as many of them are morphologically almost indistinguishable (Glaw *et al*. 2021). Although some Malagasy anurans are known to have a polymorphic coloration (Glaw & Vences 2007), in other cases subtle differences in coloration indeed help in species delimitation and diagnosis, including the presence and extension of bright color on limbs and venter (e.g., Rakotoarison *et al*. 2017; Glaw *et al*. 2020; Mullin *et al*. 2022). *Platypelis mavomavo* apparently belongs to the former group of polymorphic Malagasy anurans, contrary to expectation.

It is not currently clear if the color variation here documented for *P. mavomavo* is caused by (a) seasonal or other changes in individual coloration, or (b) true polymorphism among populations. We note that no difference was noted in coloration between males and females, and between life and reproductive stages in this study, whereas coloration was connected to sampling location, and so the latter hypothesis seems more likely. However, several frog species undergo a major color shift to yellow in the mating season, including most species of the mantellid genus *Aglyptodactylus* from Madagascar, where males become bright yellow at the height of the mating season (Glaw & Vences 2007). Such a change is not currently known among cophyline microhylids, and it seems highly unlikely also here, due to having identified ovigerous females with both gray and yellow venters, still it remains conceivable. Captive colonies or long-term monitoring of this species in the wild would help to shed light on this interesting question.

Platypelis mavomavo is among the few widespread cophyline microhylid species that seems indeed to comprise a single species, albeit with some genetic diversity across its range. Other putative examples, such as *Anodonthyla boulengerii*, *Plethodontohyla notosticta*, *Ple. inguinalis*, *Ple. ocellata*, *Platypelis grandis*, and *P. tuberifera* (Glaw & Vences 2007), are in need of taxonomic re-assessment before the same can be asserted about them. Wide-ranging

cophyline species are particularly rare in northern Madagascar, because the geographical heterogeneity appears to foster diversity and promote speciation (Wollenberg et al. 2008); other species that occur at various sites in northern Madagascar, such as *P. tsaratananaensis* (Rakotoarison *et al.* 2020) and *Stumpffia sorata* (Rakotoarison et al. 2019b), have substantial genetic diversity as well, and the former may also constitute a species complex (Rakotoarison et al. 2020). Most other cophylines in this area are microendemics (Wollenberg et al. 2008).

Platypelis mavomavo is currently considered as Endangered according to the Red List of the International Union for Conservation of Nature (IUCN 2022) because previous understanding was that its extent of occurrence (EOO) is less than 1,014 km^2, it occurs in fewer than five threat-defined locations, and there is continuing decline in the extent and quality of its habitat in northeastern Madagascar. Our new taxonomic definition of this species substantially extends both its known extent of occurrence and area of occupancy. A future new conservation assessment for the species, however, should take into account that many of the new sites are in grave danger; for instance, the tiny Andranomapanga forest fragment may already have disappeared, and Ambohitantely is a very small reserve in which a large proportion of the remaining core forest burned in 2022 bush fires. It may therefore be appropriate to classify the species as Near Threatened (NT) as it is possible that its Area of Occupancy and Extent of Occurrence could soon be substantially reduced.

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