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A new giant stream frog (genus *Mantidactylus*) from south-eastern Madagascar

(Amphibia, Mantellidae)

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We revisit the taxonomy of Madagascar's giant stream frogs of the nominal subgenus in the genus *Mantidactylus*. Based on newly collected material and extending available data sets of mitochondrial and nuclear-encoded DNA sequences, we confirm previous indications that the clade containing two mitochondrial lineages (previously named as candidate species *M.* sp. Ca66 and Ca67) concordantly differs from the other three nominal species in the subgenus by its phylogenetic position (not strongly supported as sister clade of any of the nominal species), a consistent mitochondrial divergence at a level similar to that found between other species of the subgenus (distances of 2.0–5.2 % in the 16S rRNA gene), and only limited haplotype sharing in three nuclear-encoded gene fragments. Also, the examined specimens for this clade are characterized, in comparison to other representatives of the subgenus, by smaller body size and a more distinct colour pattern on the flanks and the sides of the head, often with alternating light-dark vertical bands on the lips. We conclude that the available evidence is best reflected by recognizing *M.* sp. Ca66 as new species and we herein formally name it as *Mantidactylus lovei* sp. nov. In a preliminary way, we also include in this new species the specimens and samples belonging to the mitochondrial lineage *M.* sp. Ca67, pending further study of these genetically divergent populations. We furthermore provide preliminary evidence from archival DNA analysis confirming that the nomen *Rana pigra* Mocquard, 1900 is likely a junior synonym of *M. guttulatus*.

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Introduction

Among the Madagascan-Comoroan endemic anuran family Mantellidae, *Mantidactylus* is a species rich and morphologically highly diverse genus with currently 58 species (AmphibiaWeb 2024). *Mantidactylus* is divided into six subgenera (Glaw & Vences 2006) of which the nominal subgenus *Mantidactylus* according to the most recent revision contains three species and four additional candidate species (Rancilhac et al. 2020). *Mantidactylus* (*Mantidactylus*) are the largest mantellids, reaching sizes of up to 126 mm from snout to vent (Blommers-Schlösser & Blanc 1991, Glaw & Vences 2007, Rancilhac et al. 2020).

The species-level taxonomy of the subgenus *Mantidactylus* has proven challenging due to a rather high morphological similarity among, and morphological variation within species (Rancilhac et al. 2020), and surprisingly limited knowledge on taxonomically relevant traits such as advertisement calls or larval morphology (e. g. Vences et al. 2004, Schulze et al. 2016). A combination of "barcode fishing" from historical types, mitochondrial and nuclear DNA sequences from samples collected across Madagascar, and examination of morphology enabled Rancilhac et al. (2020) to redefine the long-confused species *M. grandidieri* Mocquard, 1895 and *M. guttulatus* (Boulenger, 1881), and to delimit and name a new species from northern Madagascar as *M. radaka* Rancilhac, Bruy, Scherz, Almeida Pereira, Preick, Straube, Lyra, Ohler, Streicher, Andreone, Crottini, Hutter, Randrianantoandro, Rakotoarison, Glaw, Hofreiter & Vences, 2020.

In addition to these three species, the subgenus currently contains four deep genetic lineages that were considered as candidate species *Mantidactylus* sp. Ca55, Ca56, Ca66 and Ca67 by Rancilhac et al. (2020), following the candidate species criteria of Vieites et al. (2009) and Perl et al. (2014). Of these, *M.* sp. Ca55 and Ca56 are only known from Betampona Reserve and Ambatoroma in the northern Central East of Madagascar (regions after Boumans et al. 2007), while *M.* sp. Ca66 and Ca67 are closely related sister lineages occurring primarily in the South East of the island, mostly at low-elevation sites.

Here, we revisit the status of these south-eastern giant *Mantidactylus* frogs based on a newly collected adult male specimen of *M.* sp. Ca66 from Manantantely and new molecular data of mitochondrial and nuclear genes. We conclude that the concordant differentiation of the south-eastern frogs in unlinked genetic markers, combined with faint but consistent morphological differences, are best reflected by their formal description as new species.

Materials and methods

This study builds upon the work of Rancilhac et al. (2020). In addition to the data published in that study, we added (i) morphological and genetic data of a new adult specimen of *M.* sp. Ca66 collected from Manantantely, (ii) DNA sequences of two additional nuclear-encoded genes, and (iii) an in-depth comparison of available morphological data of the south-eastern lineages *M.* sp. Ca66 and Ca67.

The newly analyzed specimen was collected at night along a stream, anesthetized by immersion in aqueous solutions of tricaine methanesulfonate (MS222), and subsequently euthanized by an overdose of the same substance. A tissue sample for molecular analysis was taken from the euthanized specimen and stored separately in a 1.5 ml vial filled with pure ethanol, and the voucher specimen then fixed in 95 % ethanol and preserved in 70 % ethanol. This and other, previously collected voucher specimens (see Rancilhac et al. 2020) were deposited in the Zoologische Staatssammlung München (ZSM) and the Université d'Antananarivo, Mention Zoologie et Biodiversité Animale (UADBA). In addition, specimens were examined from the Natural History Museum, London (BMNH) and the Muséum national d'Histoire naturelle, Paris (MNHN). FGZC, FGMV and ZCMV refer to field numbers of F. Glaw and M. Vences. FAZC and FN refer to field numbers of F. Andreone. APR, MSZC and ACZCV refer to field numbers of C. R. Hutter, A. P. Raselimanana, M. D. Scherz and A. Crottini, respectively. For additional isolate and specimen voucher numbers used in the phylogenetic tree herein (referring to sequences downloaded from GenBank), see Rancilhac et al. (2020). Because the paratypes in the UADBA collection have not yet been catalogued, they are here referred to with their unambiguous field numbers (i. e., UADBA-FGZC). Geographic regions within Madagascar are named according to Boumans et al. (2007) and Brown et al. (2016).

Morphometric measurements of voucher specimens were taken by MV with a manual caliper and an accuracy of 0.1 mm, as follows: snout–vent length (SVL); maximum head width (HW); head length from tip of snout to posterior edge of mouth 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 innermost edges of both nostrils (NND); forelimb length, from limb insertion to tip of longest finger (FORL); hand length, from the articulation of the carpus with the radioulna to the tip of the longest finger (HAL); hindlimb length, from the cloaca to the tip of the longest toe (HIL); foot length (FOL); foot length including tarsus (FOTL); and tibia length (TIBL). Webbing formula is reported according to Blommers-Schlösser (1979) to ensure comparability with previous species descriptions of Malagasy frogs.

For assessment of molecular divergence, DNA was extracted from tissue samples using a salt-extraction protocol (Bruford et al. 1992). We complemented the

data set of Rancilhac et al. (2020) by PCR-amplifying and sequencing the 3′-terminal fragment of the mitochondrial 16S rRNA gene (16S) using primers 16SAL (5′–CGCCTGTTTATCAAAAACAT–3′) and 16SBH-new (5′–CCTGGATTACTCCGGTCTGA–3′), modified from Palumbi et al. (1991), with cycling protocol 94°C (90 s), [94°C (45 s), 55°C (45 s), 72°C (90 s)] x 33, 72°C (300 s), and a fragment of the nuclear recombination-activating gene 1 (RAG-1) using primers Rag1-Manti-F1 (CGTGACAGAGTSAAAGGAGT) and Rag1-Manti-R1 (TCAATGATCTCTGGAACGTG) from Vences et al. (2018), plus the sequencing primer RAG1-Manti-Seq1 (5′–GCAAAGCCVTTTATTGAAACC–3′), with cycling protocol: 94°C (120 s), [94°C (20 s), 54°C (50 s), 72°C (180 s)] x 39, 72°C (600 s) (Vences et al. 2021). Furthermore, for a set of samples representing the species diversity among the subgenus *Mantidactylus*, we sequenced fragments of two additional single-copy protein-coding nuclear genes: (i) a fragment of sacsin (SACS), amplified with a nested PCR approach following Shen et al. (2012) using external primers SACSF2 (5′–AAYATHACNAAYGCNTGYTAYAA–3′) and SACSR2 (5′–GCRAARTGNCCRTTNACRTGRAA–3′) and internal primers SACSNF2 (5′–TGYTAYAAYGAYTGYCCNTGGAT–3′) and SACSNR2 (5′–CKGTGRGGYTTYTTRTARTTRTG–3′) and with cycling protocol for both PCRs: 94°C (240 s), [94°C (45 s), 45°C (40 s), 72°C (120 s)] x 45, 72°C (600 s); and (ii) a fragment of the KIAA1239 gene, with external primers KIAA1239-F1 (5′–CARCCTTGGGTNTTYCA–3′), KIAA1239-R1 (5′–CMACAAAYTGGTCRTTR–3′), and internal primers KIAA1239-NF1 (5′–GAGCCNGAYATHTTYTTYG–3′) and KIAA1239-NR1 (5′–TTCACRAANCCMCCNG–3′) (Shen et al. 2012), with nested PCR and cycling protocols as those used for SACS. PCR products were purified with Exonuclease I and Shrimp Alkaline Phosphatase digestion and sequenced by LGC Genomics (Berlin) on an automated capillary sequencer. Chromatograms were checked for base-calling errors and edited with CodonCode Aligner v 3.7.1 (Codon Code Corporation, Dedham, MA, USA) and newly determined sequences submitted to GenBank (accession numbers PV383562–PV383637 and PV386960–PV386973). Furthermore, for the holotype of *Rana pigra* (a junior synonym of *M. guttulatus*; see below) we repeated the barcode fishing procedure of Rancilhac et al. (2020) and obtained a few short read that allow for a confirmation of its synonymy. The reads were too short for inclusion in GenBank but, along with all alignments and with a metadata table of voucher specimens (including accession numbers of all other sequences) are available from the Zenodo repository (https://doi.org/10.5281/zenodo.15116210).

We aligned the 16S sequences with MAFFT v7.3 (Katoh & Stanley 2013) as implemented in Concatenator (Vences et al. 2021) using the G-INS-i option. To reconstruct evolutionary relationships in the subgenus *Mantidactylus* from a mitochondrial perspective, we inferred a Maximum Likelihood (ML) tree from the 16S alignment in IQ-Tree v.2.2.2.6 (Nguyen et al. 2015), with a substitution model selected by ModelFinder (Kalyaanamoorthy et al. 2017). Node support was assessed using 2000 full parametric bootstrap (BS) replicates (Minh et al. 2013). Uncorrected pairwise distances between sequences were calculated in MEGA7 (Kumar et al. 2016). We used ASAP (Puillandre et al. 2021) to infer and compare species partitions from the 16S data. Both for analysis of pairwise distances and for ASAP, we compiled separate trimmed alignments (available from https://doi.org/10.5281/zenodo.15116210) to minimize the effect of missing data, optimized for including as many sequences as possible for ASAP (129 sequences; 436 bp) or as many base pairs as possible for distance calculation (107 sequences; 510 bp), respectively.

For each of the three nuclear-encoded genes we used a genealogy visualization approach to graphically represent the relationship among alleles (haplotypes). Haplotypes were estimated with the PHASE algorithm (Stephens et al. 2001), and a haplotype genealogy (network) using the Fitchi approach (Matschiner 2016) was constructed in Hapsolutely (part of iTaxoTools; Vences et al. 2024).

The alignments of the three nuclear-encoded genes were analyzed independently to understand concordance (or absence thereof) in the differentiation of these three unlinked genetic markers. We follow the general lineage concept (de Queiroz 1998, 2007) in combination with a relaxed biological species criterion, i. e., demanding reproductive isolation indicated by restricted gene flow among lineages (e. g. Speybroeck et al. 2020, Dufresnes et al. 2021). Because reproductive barriers generated through time increase genealogical depth and agreement among unlinked loci (Avise & Wollenberg 1997), we use genealogical concordance (Avise & Ball 1990) between mitochondrial and nuclear loci as an indicator for restricted gene flow. This is especially relevant in populations occurring in sympatry or close geographical proximity, while keeping in mind that analyzing a small number of nuclear-encoded markers may not always yield results representative genome-scale divergence patterns. Species status is then assigned to lineages based on combined evaluation of genetic, morphological and bioacoustic evidence (Padial et al. 2010).

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new name contained herein is available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub:9B4CC676-96A4-46FD-B746-926E8A7BFDD6.

Table 1. Uncorrected pairwise distances between sequence fragments of the 16S rRNA gene (510 bp) in species and candidate species of the subgenus *Mantidactylus* (*Mantidactylus*). All values in percent. Shown is the mean distance, with minimum and maximum distances in parentheses. Grey cells are values from intraspecific comparisons.

	M. sp. Ca55	*M.* sp. Ca56	*M. grandidieri*	*M. guttulatus*	*M. radaka*	*M. lovei*
M. sp. Ca55	0.3 (0.0–0.8)					
M. sp. Ca56	3.2 (3.0–3.8)	0.0 (0.0–0.0)				
M. grandidieri	3.4 (2.8–4.2)	3.1 (2.6–3.4)	1.0 (0.0–2.2)			
M. guttulatus	2.5 (2.0–3.4)	2.5 (2.2–2.8)	2.8 (2.0–3.6)	0.2 (0.0–0.8)		
M. radaka	4.8 (4.6–5.6)	3.9 (3.8–4.8)	3.5 (3.0–4.4)	3.4 (3.0–4.2)	0.4 (0.0–2.0)	
M. lovei sp. nov.	2.8 (2.4–3.4)	3.3 (3.0–3.6)	3.1 (2.4–3.8)	2.4 (2.0–3.0)	4.3 (3.8–5.2)	0.7 (0.0–1.8)

Results

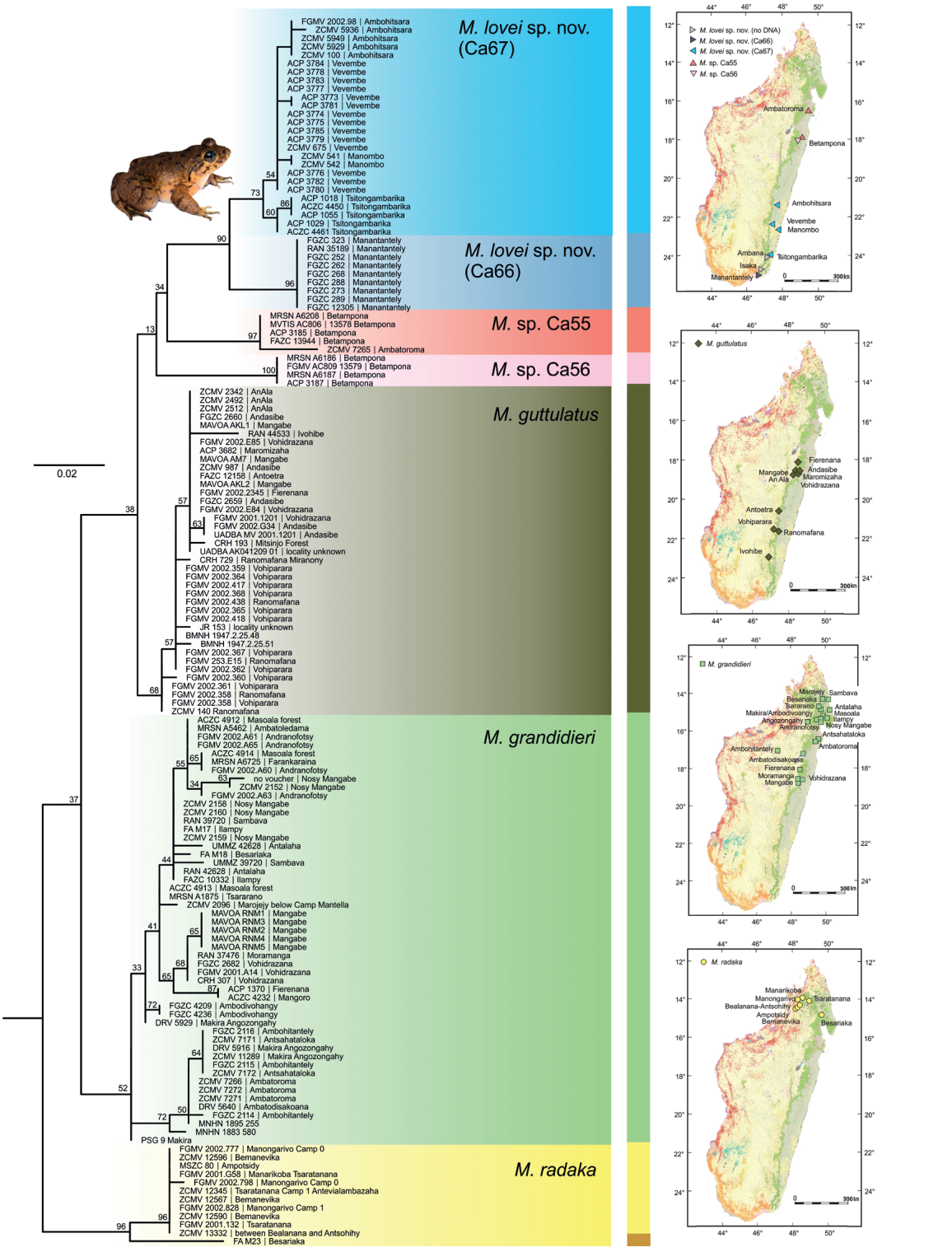
The ML tree is based on a 515 bp alignment of DNA sequences of a fragment of the mitochondrial 16S rRNA gene from 146 ingroup samples (Fig. 1). It recovered the same main clades as the analysis of Rancilhac et al. (2020) which was largely based on the same specimens. All of the recognized species and candidate species previously recognized formed highly supported mitochondrial clades (Bootstrap Support BS = 68–100 %) except for *M. guttulatus* which was only weakly supported (BS = 52 %), probably due to the inclusion of several short and incomplete sequences. The deep nodes in the tree were almost all poorly supported, confirming that the short 16S rRNA fragment is insufficient for a reliable phylogenetic resolution of the inter-species relationships. As an exception, the two previously defined candidate species and focal lineages of this study, *M.* sp. Ca66 and *M.* sp. Ca67, formed a highly supported clade (BS = 90 %).

The optimal partition inferred by ASAP suggested a species partition with eight subsets (i.e. lowest ASAP score of 2.5). These subsets fully corresponded to the current species *M. grandidieri* and *M. guttulatus*, the four candidate species (*M.* sp. Ca55, Ca56, Ca66, Ca67), and two subsets within *M. radaka* (one corresponding to a single sample from Besariaka, and the second to all other samples of the species). The second best partition (ASAP score 3.0) had an unrealistic number of 10 subsets, whereas in the third best partition (ASAP score 3.5), the two subsets corresponding to *M.* sp. Ca66 and Ca67 were merged into a single partition (detailed results available from Zenodo (https://doi.org/10.5281/zenodo.15116210). We here follow a conservative approach and favour a species partition where *M.* sp. Ca66 and Ca67 belong to the same subset which is also in agreement with allele sharing in one nuclear DNA fragment (see below). For the genetically divergent specimen from Besariaka, we continue to assign it to *M. radaka* since no nuclear DNA sequences and no further information are available from this population.

Average uncorrected pairwise 16S distances between the ASAP-defined subsets (i. e., eight species and candidate species) of the subgenus *Mantidactylus* (*Mantidactylus*) ranged from 2.4–4.8 %, with a range of all pairwise values of 2.0–5.6 % (Table 1). The highest distances were those of *M. radaka* to the other species (3.0–5.6 %) whereas low distances of 2.0–3.6 % were observed between *M. grandidieri* and *M. guttulatus*. Distances of the focal lineages (*M.* sp. Ca66 and Ca67; here merged to one lineage following the preferred species partition) to other species were 2.0–5.2 %. The distances between *M.* sp. Ca66 and Ca67 were 1.4–1.8 %.

The network obtained from phased sequences of 39 samples of the RAG-1 gene (1227 bp) did not reveal unambiguously distinct phylogroups (i. e., coherent clusters of haplotypes separated from those of other species) for several of the species and candidate species of the subgenus *Mantidactylus* (*Mantidactylus*), but allele sharing between them was exceedingly rare (Fig. 2). As in a previous analysis of the same data set (Rancilhac et al. 2020), the alleles of *M. radaka* formed a clear phylogroup separated by five mutations from all other species. Also, the vast majority of sequences of *M.* sp. Ca66 and Ca67 formed a phylogroup separated by a minimum of

Fig. 1. Maximum Likelihood tree of species in the subgenus *Mantidactylus* (*Mantidactylus*) based on a 515 bp alignment of DNA sequences of a fragment of the mitochondrial 16S rRNA gene from 146 ingroup samples. The tree was rooted based on an outgroup sequence of *Mantidactylus femoralis* (subgenus *Ochthomantis*; removed from figure to better document branch lengths within the ingroup). Numbers at nodes are bootstrap proportions in percent (2000 replicates); not shown for some of the most shallow nodes. The bars at the right of the tree indicate the eight subsets identified in the best-ranking partition of the ASAP analysis. ▷


M. lovei sp. nov. (Ca67)
FGMV 2002.98 | Ambohitsara
ZCMV 5936 | Ambohitsara
ZCMV 5949 | Ambohitsara
ZCMV 5929 | Ambohitsara
ZCMV 100 | Ambohitsara
ACP 3784 | Vevembe
ACP 3778 | Vevembe
ACP 3783 | Vevembe
ACP 3777 | Vevembe
ACP 3773 | Vevembe
ACP 3781 | Vevembe
ACP 3774 | Vevembe
ACP 3775 | Vevembe
ACP 3785 | Vevembe
ACP 3779 | Vevembe
ZCMV 675 | Vevembe
ZCMV 541 | Manombo
ZCMV 542 | Manombo
ACP 3776 | Vevembe
ACP 3782 | Vevembe
ACP 3780 | Vevembe
ACP 1018 | Tsitongambarika
ACZC 4450 | Tsitongambarika
ACP 1055 | Tsitongambarika
ACP 1029 | Tsitongambarika
ACZC 4461 Tsitongambarika
M. lovei sp. nov. (Ca66)
FGZC 323 | Manantantely
RAN 35189 | Manantantely
FGZC 252 | Manantantely
FGZC 262 | Manantantely
FGZC 268 | Manantantely
FGZC 288 | Manantantely
FGZC 273 | Manantantely
FGZC 289 | Manantantely
FGZC 12305 | Manantantely
M. sp. Ca55
MRSN A6208 | Betampona
MVTIS AC806 | 13578 Betampona
ACP 3185 | Betampona
FAZC 13944 | Betampona
ZCMV 7265 | Ambatoroma
M. sp. Ca56
MRSN A6186 | Betampona
FGMV AC809 13579 | Betampona
MRSN A6187 | Betampona
ACP 3187 | Betampona
M. guttulatus
ZCMV 2342 | AnAla
ZCMV 2492 | AnAla
ZCMV 2512 | AnAla
FGZC 2660 | Andasibe
MAVOA AKL1 | Mangabe
RAN 44533 | Ivohibe
FGMV 2002.E85 | Vohidrazana
ACP 3682 | Maromizaha
MAVOA AM7 | Mangabe
ZCMV 987 | Andasibe
FAZC 12158 | Antoetra
MAVOA AKL2 | Mangabe
FGMV 2002.2345 | Fierenana
FGZC 2659 | Andasibe
FGMV 2002.E84 | Vohidrazana
FGMV 2001.1201 | Vohidrazana
FGMV 2002.G34 | Andasibe
UADBA MV 2001.1201 | Andasibe
CRH 193 | Mitsinjo Forest
UADBA AK041209 01 | locality unknown
CRH 729 | Ranomafana Miranony
FGMV 2002.359 | Vohiparara
FGMV 2002.364 | Vohiparara
FGMV 2002.417 | Vohiparara
FGMV 2002.368 | Vohiparara
FGMV 2002.438 | Ranomafana
FGMV 2002.365 | Vohiparara
FGMV 2002.418 | Vohiparara
JR 153 | locality unknown
BMNH 1947.2.25.48
BMNH 1947.2.25.51
FGMV 2002.367 | Vohiparara
FGMV 253.E15 | Ranomafana
FGMV 2002.362 | Vohiparara
FGMV 2002.360 | Vohiparara
FGMV 2002.361 | Vohiparara
FGMV 2002.358 | Ranomafana
FGMV 2002.358 | Vohiparara
ZCMV 140 Ranomafana
M. grandidieri
ACZC 4912 | Masoala forest
MRSN A562 | Ambatoledama
FGMV 2002.A61 | Andranofotsy
FGMV 2002.A65 | Andranofotsy
ACZC 4914 | Masoala forest
MRSN A6725 | Farankaraina
FGMV 2002.A60 | Andranofotsy
no voucher | Nosy Mangabe
ZCMV 2152 | Nosy Mangabe
FGMV 2002.A63 | Andranofotsy
ZCMV 2158 | Nosy Mangabe
ZCMV 2160 | Nosy Mangabe
RAN 39720 | Sambava
FA M17 | Ilampy
ZCMV 2159 | Nosy Mangabe
UMMZ 42628 | Antalaha
FA M18 | Besariaka
UMMZ 39720 | Sambava
RAN 42628 | Antalaha
FAZC 10332 | Ilampy
ACZC 4913 | Masoala forest
MRSN A1875 | Tsararano
ZCMV 2096 | Marojejy below Camp Mantella
MAVOA RNM1 | Mangabe
MAVOA RNM3 | Mangabe
MAVOA RNM2 | Mangabe
MAVOA RNM4 | Mangabe
MAVOA RNM5 | Mangabe
RAN 37476 | Moramanga
FGZC 2682 | Vohidrazana
FGMV 2001.A14 | Vohidrazana
CRH 307 | Vohidrazana
ACP 1370 | Fierenana
ACZC 4232 | Mangoro
FGZC 4209 | Ambodivohangy
FGZC 4236 | Ambodivohangy
DRV 5929 | Makira Angozongahy
FGZC 2116 | Ambohitantely
ZCMV 7171 | Antsahataloka
DRV 5916 | Makira Angozongahy
ZCMV 11289 | Makira Angozongahy
FGZC 2115 | Ambohitantely
ZCMV 7172 | Antsahataloka
ZCMV 7266 | Ambatoroma
ZCMV 7272 | Ambatoroma
ZCMV 7271 | Ambatoroma
DRV 5640 | Ambatodisakoana
FGZC 2114 | Ambohitantely
MNHN 1895 255
MNHN 1883 580
PSG 9 Makira
M. radaka
FGMV 2002.777 | Manongarivo Camp 0
ZCMV 12596 | Bemanevika
MSZC 80 | Ampotsidy
FGMV 2001.G58 | Manarikoba Tsaratanana
FGMV 2002.798 | Manongarivo Camp 0
ZCMV 12345 | Tsaratanana Camp 1 Antevialambazaha
ZCMV 12567 | Bemanevika
FGMV 2002.828 | Manongarivo Camp 1
ZCMV 12590 | Bemanevika
FGMV 2001.132 | Tsaratanana
ZCMV 13332 | between Bealanana and Antsohihy
FA M23 | Besariaka
0.02
M. lovei sp. nov. (no DNA)
M. lovei sp. nov. (Ca66)
M. lovei sp. nov. (Ca67)
M. sp. Ca55
M. sp. Ca56
Ambatoroma
Betampona
Ambohitsara
Vevembe
Manombo
Ambana
Tsitongambarika
Isaka
Manantantely
M. guttulatus
Fierenana
Andasibe
Mangabe
An'Ala
Maromizaha
Vohidrazana
Antoetra
Vohiparara
Ranomafana
Ivohibe
M. grandidieri
Marojejy
Sambava
Besaraka
Tsararano
Antalaha
Masoala
Makira/Ambodivoangy
Ilampy
Angozongahy
Nosy Mangabe
Andranofotsy
Antsahataloka
Ambohitantely
Ambatoroma
Ambatodisakoana
Fierenana
Moramanga
Vohidrazana
Mangabe
M. radaka
Manarikoba
Manongarivo
Saratanana
Bealanana-Antsohihy
Ampotsidy
Bemanevika
Besariaka
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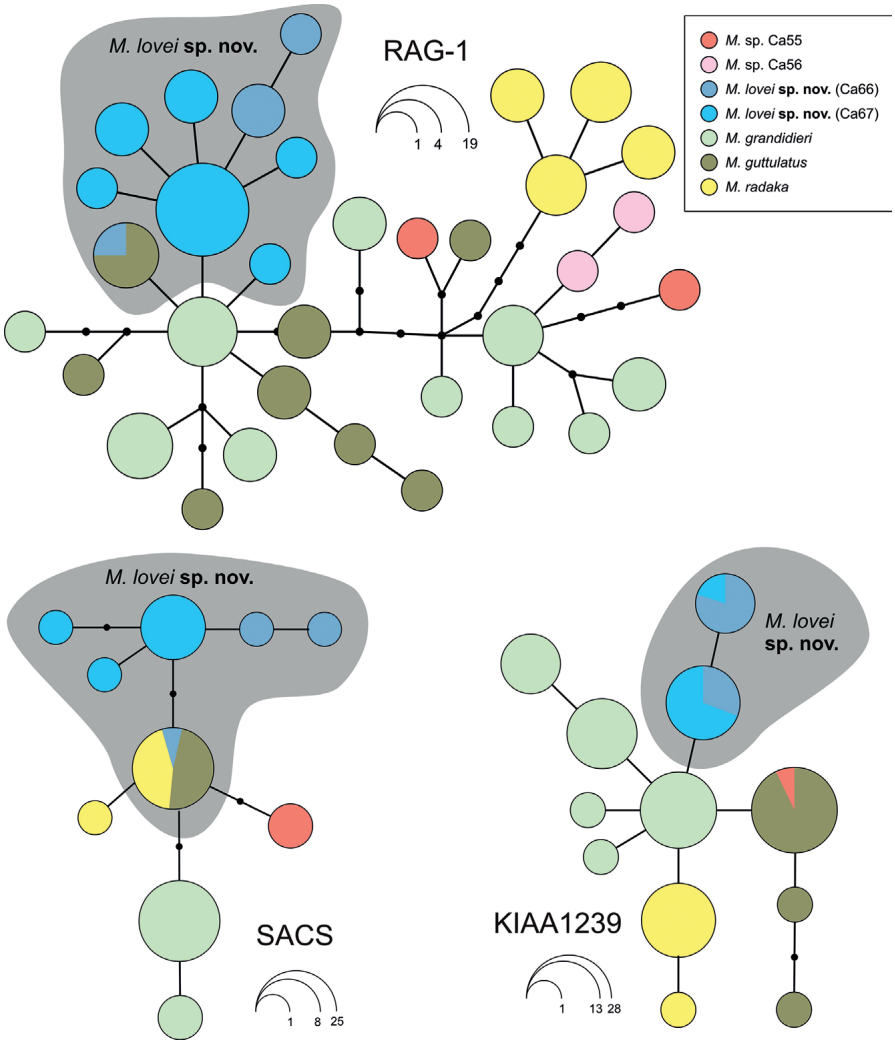


Fig. 2. Haplotype genealogies for three nuclear-encoded protein-coding single-copy genes in species of the subgenus *Mantidactylus* (*Mantidactylus*). The networks are based on sequences of 39 samples for RAG-1 (1227 bp), 33 samples for SACS (904 bp) and 47 samples for KIAA1239 (763 bp). Sequences were phased before analysis (thus, every network is based on twice the number of sequences compared to sample number). The underlying grey shapes summarize the alleles found in the two lineages (*M.* sp. Ca66 and *M.* sp. Ca67) that herein are together considered as belonging to the new species, *Mantidactylus lovei* sp. nov.

one mutation from the other species, except for two sequences, one of which was shared with *M. guttulatus*. The datasets of the phased DNA fragments of SACS (33 samples; 904 base pairs) and KIAA1239 (47 samples; 763 base pairs) also supported a separation of *M.* sp. Ca66 + Ca67 from other species (Fig. 2). In KIAA1239, the Ca66 + Ca67 formed a cluster and both shared two alleles that differed by one mutation from the nearest haplotype. In SACS, the corresponding phylogroup was separated by two mutations from the other species, but one specimen of *M.* sp. Ca66 shared an allele with specimens of *M. guttulatus* and *M. grandidieri*.

The geographically closest known contact between the *M.* sp. Ca66 + Ca67 lineage and another species of the subgenus is with *M. guttulatus* in the Ranomafana area. Here, *M. guttulatus* occurs in Ranomafana National Park whereas *M.* sp. Ca67 was found in a forest fragment near Ambohitsara. The haplotype analyses, however, did not reveal a particular signal

of admixture among these geographically adjacent populations. In RAG-1, the observed haplotype sharing occurred between *M.* sp. Ca66 and geographically distant specimens of *M. guttulatus* from Vohidrazana; and likewise in the KIAA1239 network, the central haplotype was shared between specimens of *M. guttulatus*, *M. radaka* and the geographically distant *M.* sp. Ca66 from Manantantely, suggesting incomplete lineage sorting.

Morphologically, species in the nominal subgenus of *Mantidactylus* are known to be rather similar to each other and to differ by subtle characters only. Since calling males have been found of one species only (Vences et al. 2004) and femoral glands can be recognized already in recently metamorphosed juveniles and (in some species) in females, it furthermore is sometimes difficult to unambiguously identify sexually mature adults. In specimens of *M.* sp. Ca66 and Ca67, distinct femoral glands are present in females as well as males, and the smaller size of femoral glands of females only becomes obvious by direct comparison. This is an immediate difference to *M. radaka* which also differs by several other characters (see Diagnosis below). By direct comparison with preserved material and with measurements published in Rancilhac et al. (2020), it becomes apparent that specimens of *M.* sp. Ca66 and Ca67 appear to be relatively small sized, without any really giant individual so far known from their known range. Adult genotyped individuals have SVLs of 76–77 mm in males and 86 mm in one female (Table 2). Additional specimens from the MNHN collection (not genotyped; approximate body sizes from photographs with scales) from Ambana (series MNHN-RA 1973.877–881) and Isaka-Ivondro (MNHN-RA 1935.169) fully agree with this assessment, with ca. 75 mm SVL in three males with large femoral glands, and ca. 90 mm in one female. Although specimens of *M.* sp. Ca66 and Ca67 may attain larger sizes, we thus hypothesize that on average they remain relatively small in most populations. In contrast, males of *M. grandidieri* and *M. guttulatus* regularly reach larger body sizes of 88–98 mm (measurements in Rancilhac et al. 2020); even up to 118 mm if the holotype of the nomen *Rana pigra* Mocquard, 1900 (MNHN-RA 1899.410), a synonym of *M. guttulatus* (see below), is indeed a male. A further distinctive character of the majority of specimens of *M.* sp. Ca66 and Ca67 is the pattern on the sides of the head, often with alternating light-dark vertical bands on the lips (Fig. 3). Along with some other subtle morphological differences detailed in the diagnosis below, this suggests a weak but consistent morphological differentiation of the *M.* sp. Ca66 + Ca67 clade from other species in the same subgenus.

Table 2. Morphometric measurements (in mm) of holotype (HT), two paratypes (PT) and one additional specimen of *Mantidactylus lovei* sp. nov. M, male; F, female; SA, subadult. See Materials and methods for other abbreviations.

Catalogue number	ZSM 176/2004	ZSM 135/2023	ZSM 155/2004	ZSM 2410/2007
Field number	FGZC 323	FGZC 12305	FGZC 288	ZCMV 5929
Locality	Manantantely	Manantantely	Manantantely	Ambohitsara
Status	HT	PT	PT	–
Lineage	"Ca66"	"Ca66"	"Ca66"	"Ca67"
Sex	M	M	F	SA
SVL	77.3	76.4	86.0	56.9
HW	33.4	34.9	35.2	22.5
HL	32.3	32.2	36.0	23.7
TD	5.4	3.7	5.2	4.2
ED	10.0	10.7	12.2	7.5
END	5.8	6.2	6.0	4.5
NSD	4.3	5.7	5.4	3.5
NND	7.2	7.1	7.4	6.0
FORL	41.0	41.8	45.7	30.4
HAL	21.8	21.3	26.8	14.7
HIL	115.7	115.0	127.5	82.4
FOTL	55.3	53.2	56.5	36.6
FOL	39.0	38.1	40.2	25.3
TIBL	36.3	37.7	39.8	25.2
FGL	6.9	6.5	5.5	4.4
FGW	5.3	5.3	3.9	2.2

Taken together, the molecular and morphological evidence suggest consistent differences of the populations previously assigned to the candidate species *M.* sp. Ca66 and Ca67 to the other three species in the subgenus *Mantidactylus* (*Mantidactylus*). The amount of mitochondrial divergence is at the same level as between other (partly syntopic) species of the subgenus, there is only a limited amount of haplotype sharing in the three nuclear genes studied (including with populations of *M. guttulatus* living in close geographical distance in the Ranomafana area), and there are subtle but consistent morphological differences to the other three species. Phylogenetically, the *M.* sp. Ca66+Ca67 clade is sister to two other candidate species, but not strongly supported as sister lineage to a nominal species in which it could be potentially included as deep conspecific lineage. At the same time, no obvious morphological differentiation was observed between specimens of the two mitochondrial lineages Ca66 and Ca67, these two lineages formed a highly supported mitochondrial clade, were part of the same phylogroup in all three nuclear genes and shared haplotypes in one of them, and they differed by <2% in the 16S fragment. To accommodate this pattern, we propose to consider the populations belonging to the lineages Ca66 and Ca67 together as a separate species level lineage which we formally name and describe in the following.

Mantidactylus lovei sp. nov.

Figs 3–4

ZooBank LSID: urn:lsid:zoobank.org:act:5B3D7620-3A41-4B33-84FE-9B49F3B90F43

Holotype. ZSM 176/2004 (field number FGZC 323), adult male, collected by F. Glaw, M. Puente, M. Teschke née Thomas and R.D. Randrianiaina on 8 February 2004 at Manantantely (24°59'S, 46°55'E, between 20–150 m a.s.l.), southeastern Madagascar.

Paratypes. Seven specimens, all from southeastern Madagascar: ZSM 155/2004 (FGZC 288), adult female with same collection data as holotype. ZSM 139/2004 (FGZC 262), with same collectors and locality as holotype but collected on 7 February 2004. UADBA-FGZC 289, specimen of unknown sex and maturity, with same collection data as holotype; UADBA-FGZC 252, UADBA-FGZC 268, UADBA-FGZC 273, three specimens of unknown sex and maturity with same collectors and locality as holotype but collected on 7 February 2004. ZSM 135/2023 (FGZC 12305), adult male collected by M. Vences, J.B. Ramanamanjato and S. Rasamison on 11 November 2023 at Manantantely.

Additional material examined. ZSM 2410/2007 (ZCMV 5929), collected by M. Vences, K.C. Wollenberg and E. Rajeriarison on 3 March 2007 at Ambohitsara (-21.3572, 47.8157); belonging to genetic lineage "Ca67" (not "Ca66" as specimens from the type locality) and therefore not included in paratype series. MNHN-RA 1935.169, male, collected at Isaka-Ivondro at an unknown date by René Catala and donated to the Paris museum in January 1935; and MNHN-RA 1973.877–881, two males, one female and two probably subadult specimens, collected in 1972 at Ambana by C.P. Blanc; not genotyped and therefore not included in the type series.

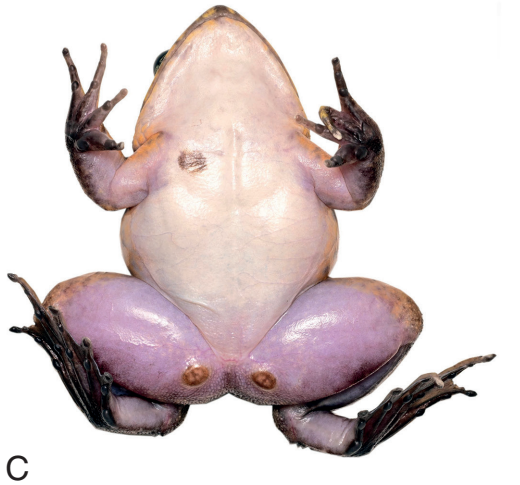
Diagnosis. The new species is assigned to the genus *Mantidactylus* based on the presence of an intercalary element between terminal and subterminal phalanges of fingers and toes (verified by external observation only), of femoral glands with a central depression in males and of rudimentary femoral glands in females. Within *Mantidactylus*, it is assigned to the nominal subgenus *Mantidactylus* by combination of (1) large body size (known male SVL 76–77 mm), (2) absence of dorsolateral colour border, (3) absence of a distinct frenal stripe, (4) absence of large yellowish patches or stripes in the inguinal region or between coloration of flanks and belly, (5) extensively webbed feet and (6) riparian habits, living very close to or in streams. The assignment of the species to this group is also supported by its molecular phylogenetic relationships.

From the three described species in the subgenus *Mantidactylus*, the new species is distinguished as follows: From *M. radaka* by a distinct supratympanic fold and clearly visible tympanum (vs. often-hidden tympanum) and larger horizontal tympanum diameter (male TD/SVL 0.048-0.079 vs. 0.037–0.042), smaller femoral glands in males (FGL/SVL 0.085-0.089 vs. 0.133–0.195) and femoral glands visible in females (vs. not recognizable), probably smaller body size of most specimens (known male SVL

Fig. 3. *Mantidactylus lovei* sp. nov., images of specimens in life. **A, B, C.** Male paratype ZSM 135/2023 from Manantantely in dorsolateral, dorsal and ventral views, photographed in November 2023. **D.** Paratype from Manantantely (probably corresponding to one of the UADBA specimens) in dorsolateral view, photographed in 2004. **E.** juvenile (not genotyped) from Nahampoana in dorsolateral view, tentatively assigned to this species, photographed in 1994; **F.** specimen from Vevembe forest (not assignable to a voucher specimen), belonging to lineage Ca67, in dorsolateral view **G.** Specimen FAZC 15332 from Tsitongambarika, belonging to lineage Ca67, in dorsolateral view. Photographs in panels F and G by A. Crottini and F. Andreone, and taken from Rancilhac et al. (2020). Images not to scale. ▷


A


B


C


D


E


F


G

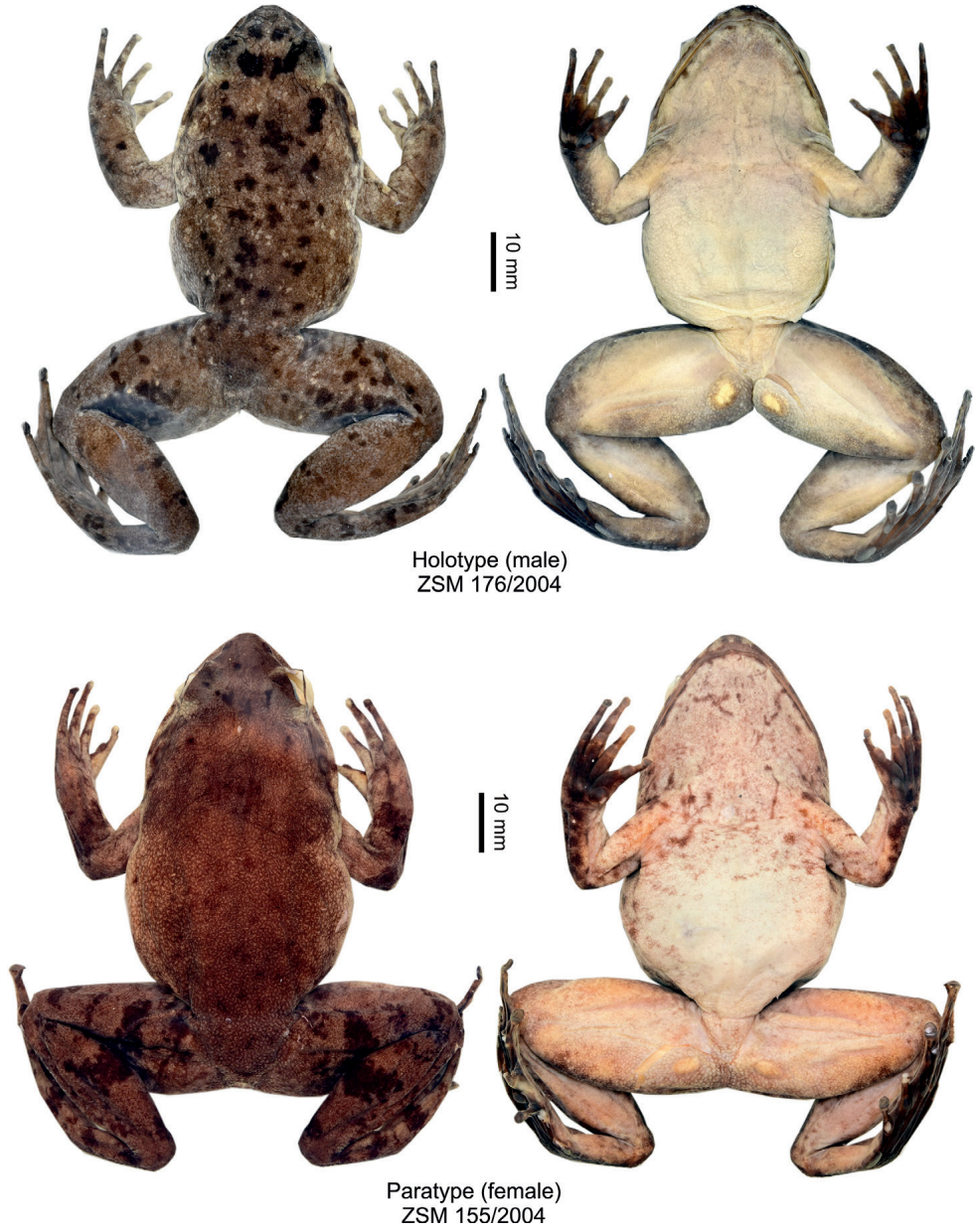


Fig. 4. Preserved male holotype (ZSM 176/2004, field number FGZC 323) and female paratype ZSM 155/2004 (FGZC 288) of *Mantidactylus lovei* sp. nov. from Manantantely in dorsal and ventral views.

76–77 mm vs. 88–93 mm), shagreened or weakly granular dorsal skin in life and almost smooth skin in preservative (vs. strongly granular dorsally), and head with distinct dark-light pattern, often forming alternating vertical bands on upper and/or lower lip (vs. without lateral pattern); from *M. guttulatus*, by probably smaller body size of most specimens (known male SVL 76–77 mm vs. 90–98 mm), slightly larger male femoral glands (FGL/SVL 0.085–0.089 vs. 0.059–0.079), mostly relatively longer hindlimbs (HIL/SVL 1.45–1.51 vs. 1.28–1.49, with only one out of 11 individuals >1.43; Rancilhac et al. 2020), and head with distinct dark-light pattern, often forming alternating vertical bands on upper and/or lower lip

(vs. without lateral pattern); most similar in body proportions to *M. grandidieri* but differing by probably slightly smaller body size of most specimens (known male SVL 76–77 mm vs. 81–88 mm), and head with distinct dark-light pattern, often forming alternating vertical bands on upper and/or lower lip (vs. without lateral pattern).

Description of the holotype

Adult male in excellent state of preservation (Fig. 4). Femoral gland on left shank cut open and skin inflexed for internal examination; a transverse and a longitudinal cut on abdomen for gonad examination, part of the tongue taken as tissue sample. For measurements, see Table 2. Body relatively stout. Head wider than long, slightly wider than body. Snout blunt. Nostrils directed dorsally, slightly protuberant, nearer to tip of snout than to eye. Canthus rostralis moderately distinct, loreal region concave. Upper part of the tympanum hidden under the tympanic fold. Tympanum medium-sized, horizonal diameter of tympanum 54 % of horizontal eye diameter. Supratympanic fold distinct, regularly curving from eye to axilla by forming a 90° arc. Tongue ovoid, probably bifid distally (not fully visible due to tissue sampling). Vomerine teeth form two aggregations, positioned posterolateral to choanae and quite close to each other medially, in square format, with small and sharp tooth serrations posteriorly (seven on the right, and five on the left). Maxillary teeth present. Choanae ovoid, almost slit-like. Subarticular tubercles single. Inner and outer metacarpal tubercle present; inner tubercle distinct, outer tubercle flat but recognizable by different coloration. Fingers without webbing. Relative length of fingers: I < II < IV < III. Finger discs very slightly enlarged. Nuptial pads absent. Foot longer than tibia (110 %). Lateral metatarsalia separated by webbing. Inner metatarsal tubercle present and distinct. Outer metatarsal tubercle not recognisable. Webbing formula: 1(0), 2i(1), 2e(0), 3i(1), 3e(0), 4i(1), 4e(0.5), 5(0). Relative length of toes: I < II < III = V < IV. Skin on the upper surface regularly shagreened over the entire dorsal surface. Ventral side smooth. Femoral glands (type 4 according to Glaw et al. 2000) distinct in external view, with clear central depression, consisting of only four large granules of up to ca. 1 mm in diameter visible in internal view. Colour in preservative (after 19 years in preservative) dorsally brown, covered with numerous irregular and poorly delimited darker brown patches and some smaller interspersed beige dots. Supratympanic fold and tympanum are dark brown. A dark brown bar between the eyes, and density of dark markings is higher on the snout. Supralabial area, especially on the left side irregularly marked with 3–4 alternating dark brown vs. three light greyish markings. Flanks are relatively light coloured. Venter light cream, slightly darker on hindlimbs where some dark pigment is present. Coloration in life not recorded.

Colour in life. Based on paratype ZSM 135/2023. Dorsal surface brownish with poorly contrasted but sharply delimited large dark brown spots. Three dark crossbands on each shank and thigh, and two dark crossbands on lower forelimb. Flanks, lateral sides of head underneath the eye and the supratympanic fold, as well as proximal part of forelimb and a small area posterodorsal of eye are lighter yellowish brown, with a contrasting but irregular pattern of large dark spots and markings, forming an irregular dark-light pattern on lips. Ventrally uniformly pinkish on limbs and non-transparent whitish on belly, chest and throat.

Variation. In the female paratype ZSM 155/2004, the femoral gland in internal view consists of ca. 44 gland granules of up to ca. 1 mm in diameter. According to the available photographs of live specimens (Fig. 3) a light yellowish-brown lateral colour, on flanks and on the sides of the head, is typical for all individuals. In one further specimen from Manantantely (Fig. 3D) and one specimen from Tsitongambarika (Fig. 3G) also the well-delimited dark markings laterally on the head are quite distinct, whereas in one specimen from Vevembe, they are missing and the area of the lips is rather uniformly light yellowish brown.

Etymology. The species is dedicated to reptile enthusiast, photographer and author Bill Love, in recognition of his contributions to the knowledge of natural history and herpetoculture of Madagascar's amphibians and reptiles.

Available earlier names. The nomen *Rana pigra* Mocquard, 1900, based on the holotype MNHN-RA 1899.410 from 'forêt d'Ikongo' is currently considered as a synonym of *M. guttulatus,* but its type locality is in the South East and therefore well within the general range of both *M. guttulatus* and *M. lovei.* It therefore must be considered as possible earlier available name for *M. lovei.* According to the measurements provided by Rancilhac et al. (2020), the holotype of *pigra* is a male specimen of very large size (SVL 118.8 mm), much larger than the known material of *M. lovei*, and its relative hindlimb length (ratio HIL/SVL 1.37) fits with *M. guttulatus* and is lower than the values recorded from *M. lovei.* Rancilhac et al. (2020) stated that only a few reads could be obtained by targeted enrichment from the holotype, and did not provide further analyses of these. Based on a subsequent repeat of the baiting and sequencing, using the exact same methods as described at length in Rancilhac et al. (2020), a few more fragments could be obtained for two mitochondrial genes: 16S (177 bp) and cytochrome *b* (77 bp). A comparison of these with

sequences of *M. guttulatus* and *M. lovei* revealed three diagnostic substitutions in 16S in which the *pigra* sequences agreed with *M. guttulatus* but differed from *M. lovei*, and a 100 % agreement of a recovered short cytochrome *b* fragment with *M. guttulatus* (alignments available from the Zenodo repository: https://doi.org/10.5281/zenodo.15116210). Based on this combined evidence we conclude that *Rana pigra* Mocquard, 1900 should be continued to be regarded as junior synonym of *M. guttulatus*, and is not an earlier name for the lineage here named *M. lovei.*

Natural History. The new species has exclusively been found in clear forest streams in intact or degraded low-elevation rainforest. Specimens were observed at night, sitting in or next to the water. Advertisement calls, tadpoles and reproductive habits unknown.

Distribution. Specimens unambiguously belonging to this species, i. e., of lineage Ca66, in a strict sense, are only known from (1) the type locality, Manantantely close to Tolagnaro in the extreme South-East of Madagasca. This also includes specimen UMMZ 197846 (field number RAN 35189), which has been collected at Manantantely forest as well according to the online UMMZ database. Specimens of the divergent mitochondrial lineage Ca67 which we consider as conspecific with the Manantantely samples are known from (2) Tsitongambarika, (3) Vevembe, (3) Manombo, and (4) Ambohitsara (see also Rancilhac et al. 2020). The species' elevational range is from < 100 m a. s. l. at Manombo to 500–600 m a. s. l. at Vevembe.

Discussion

In this study we expanded the available molecular and morphological data on populations of the subgenus *Mantidactylus* (*Mantidactylus*) in the South East of Madagascar, thereby providing support for the hypothesis according to which they differ at the species level from other lineages in this subgenus. After the description of *M. lovei,* the subgenus now consists of four nominal species, plus up to four mitochondrial lineages of uncertain status (Fig. 1 and Rancilhac et al. 2020). For a full taxonomic inventory of the subgenus, it will be of importance to investigate the lineages *M.* sp. Ca55 and Ca56 which both co-occur in the Betampona Special Reserve in the Northern Central East of Madagascar, as well as populations of *M. radaka* from the North East of Madagascar (here represented by one sample from Besariaka). For these three lineages, currently, only a limited number of tissue samples is available, and no voucher specimens have been morphologically examined. While in-depth analysis of the available materials, e. g. by genomic approaches may yield some further insights especially for the two Betampona lineages, a conclusive taxonomic resolution and possible formal description of these taxa will probably require collection of new voucher specimens.

A further mitochondrial lineage is *M.* sp. Ca67 which herein is considered a deep conspecific lineage within *M. lovei.* This is based on molecular data which do not show a convincing and consistent differentiation of Ca67 in the nuclear-encoded genes and due to the low level of mitochondrial distances from Ca66 in the 16S gene fragment (1.4–1.8 %). Very few voucher specimens of the Ca67 lineage are available for examination, and only a single subadult could be measured for the present study (ZSM 2410/2007 from Ambohitsara). We therefore propose to include *M.* sp. Ca67 as deep conspecific lineage in *M. lovei*, and different from other recent studies on *Mantidactylus* (e. g. Scherz et al. 2022) refrain from formally naming Ca67 as subspecies of *M. lovei* due to the scarcity of preserved adult specimens that could serve to understand its possible morphological differentiation. For a final clarification of the status of this lineage, additional material is necessary and ideally, the contact zone between Ca66 and Ca67 in the South East of Madagascar should be mapped and possible hybridization or absence thereof quantified.

Species in the subgenus *Mantidactylus* (*Mantidactylus*) have very large body sizes compared to most other native Malagasy anurans (Blommers-Schlösser & Blanc 1991, Glaw & Vences 2007). The subgenus contains at least two apparently range-restricted mitochondrial lineages, i. e., the lineage Ca66 within *M. lovei* which is only known from Manantantely; and *M.* sp. Ca56 which is only known from Betampona. Microendemism, that is, the existence of species with very small ranges (Wilmé et al. 2006, Brown et al. 2016) is common among Madagascar's biota, but should be rare in species with large body sizes which statistically have larger ranges (Pabijan et al. 2012, Brown et al. 2016). It is possible that species restricted to lowlands show limited gene flow over large rivers which are wider at low elevations than in headwater areas (e. g. Gehring et al. 2012); to which degree this hypothesis applies to Madagascar's herpetofauna is worth more comprehensive testing.

Independent of the status of the two mitochondrial lineages here subsumed under *M. lovei*, i. e., Ca66 and Ca67, both clearly form a monophyletic group and are geographically well-delimited to the South East and reaching into the southern Central East zones according to the zonation of Boumans et al. (2007). Here, they appear to mostly occur at low-elevational sites whereas *M. guttulatus* is found at higher elevations (see inset maps in Fig. 1). The species morphologically closest to *M. lovei* accord-

ing to currently available data is *M. grandidieri*, which also predominantly occurs in lowlands in the northern Central East and North East, although it also reaches an elevation of 1009 m a. s. l. at Angozongahy at the western edge of Makira Reserve and 1500–1600 m a. s. l. in Ambohitantely Special Reserve. Due to the almost complete deforestation at low elevations between the known ranges of *M. lovei* and *M. grandidieri*, it is uncertain where the contact zone between these two species is or has been. A continuation of surveys of rainforest fragments in these areas (Gehring et al. 2010) may yield remnant populations to answer these biogeographic questions. According to the 16S trees presented here (Fig. 1) and in Rancilhac et al. (2020), it is uncertain whether *M. grandidieri* and *M. lovei* may be vicariant sister species, given the poor support of deep nodes in the phylogeny. Additional mitochondrial genes for all species of the subgenus may yield higher support for these nodes and thus better clarify their phylogenetic relationships, as in a previous study which however only contained three species of the subgenus (Wollenberg et al. 2011).

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