Sympatric lineages in the *Mantidactylus ambreensis* complex of Malagasy frogs originated allopatrically rather than by in-situ speciation

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ABSTRACT

Madagascar’s biota is characterized by a high degree of microendemism at different taxonomic levels, but how colonization and in-situ speciation contribute to the assembly of local species communities has rarely been studied on this island. Here we analyze the phylogenetic relationships of riparian frogs of the *Mantidactylus ambreensis* species complex, which is distributed in the north of Madagascar and was originally described from Montagne d’Ambre, an isolated mountain of volcanic origin, currently protected within Montagne d’Ambre National Park (MANP). Data from mitochondrial DNA, and phylogenomic data from FrogCap, a sequence capture method, independently confirm that this species complex is monophyletic within the subgenus *Ochthomantis*, and identify two main clades within it. These two clades are separated by 5.6–6.8% pairwise distance in the mitochondrial 16S rRNA gene and co-occur in MANP, with one distributed at high elevations (940–1375 m a.s.l.) and the other at lower elevations (535–1010 m a.s.l.), but show almost no haplotype sharing in the nuclear RAG1 gene. This occurrence in syntopy without admixture confirms them as independent evolutionary lineages that merit recognition as separate species, and we here refer to them as high-elevation (HE) and low-elevation (LE) lineage; they will warrant taxonomic assessment to confidently assign the name *ambreensis* to one or the other. Populations of the *M. ambreensis* complex from elsewhere in northern Madagascar all belong to the LE lineage, although they do occur over a larger elevational range than in Montagne d’Ambre (285–1040 m a.s.l.). Within LE there are several phylogroups (LE1–LE4) of moderately deep divergence (1.5–2.8% in 16S), but phylogroup LE4 that occurs in MANP has a deeply nested phylogenetic position, as recovered separately by mitochondrial and sequence capture datasets. This suggests that HE and LE did not diverge by a local fission of lower and upper populations, but instead arose through a more complex biogeographic scenario. The branching pattern of phylogroups LE1–LE4 shows a clear south-to-north phylogeographic pattern. We derive from these results a testable hypothesis of vicariant speciation that restricted the HE lineage to MANP and the LE candidate species to a climatic refugium further south, with subsequent northwards range expansion and secondary colonization of MANP by LE. These results provide an example for complex assembly of local microendemic amphibian faunas on Madagascar.

1. Introduction

Madagascar’s vast size and isolation in deep geological time led to an exceptional proportion of its fauna and flora being endemic (Myers et al., 2000; Vences, 2004; Wilmé et al., 2006; Yoder and Nowak, 2006). Many of the endemic clades of organisms in Madagascar probably originated by Cenozoic transmarine dispersal from Africa and in some cases Asia, and subsequently diversified in adaptive or non-adaptive radiations on the island (Vences, 2004; Yoder and Nowak, 2006; Pouz et al., 2008; Crottini et al., 2012; Samonds et al., 2012,
2013; Scantlebury, 2013; Warren et al., 2010; Wollenberg et al., 2017; Yuan et al., 2019). Their diversification on Madagascar was probably facilitated by the regionally and locally pronounced environmental gradients characterizing the island.

As one area of particularly obvious environmental gradients, Montagne d’Ambre National Park (MANP) merits special attention due to three major characteristics that may constitute drivers of species formation through various processes, from geographic isolation to ecological speciation at ecotones. First, MANP protects a massif with humid forest at higher elevations surrounded by drier lowland habitat, and this forest-cover structure, topography, and geomorphology is of rather ancient Tertiary origin (Du Puy and Moat, 2003). Currently isolated from the large block of eastern rainforest further to the south, the mountain was temporarily connected to other forest blocks in former times (Ramanananjato et al., 1999; Vences et al., 2003; D’Cruze et al., 2006, 2007). The humid tropical microclimate of the upper portions of Montagne d’Ambre further reinforces its isolation from the surrounding dry deciduous forests with notably less mesic conditions (Nicoll and Langrand, 1998; Battistini, 1996; GOODMAN et al., 1996; D’Cruze et al., 2008) has led to substantial habitat differences connected by gradual bioclimatic transitions. Third, volcanic eruptions on the massif occurred from the Miocene to Holocene and during certain periods of activity resulted in regional denudation of vegetation, such as during the Plio-Quaternary (Rossi, 1974; Battistini, 1996; Goodman et al., 1996; D’Cruze et al., 2008) has led to substantial habitat differences connected by gradual bioclimatic transitions. Third, volcanic eruptions on the massif occurred from the Miocene to Holocene and during certain periods of activity resulted in regional denudation of vegetation, such as during the Plio-Quaternary (Rossi, 1974; Battistini, 1996; Goodman et al., 1996; D’Cruze et al., 2008). As far as available, sample names include elevation for specimens from Montagne d’Ambre National Park (MANP). Numbers at nodes are Bayesian posterior probabilities.

Fig. 1. (A) Bayesian Inference phylogenetic tree of 80 samples of Mantidactylus ambreensis based on analysis of a segment of the 16S rRNA gene (520 bp) for all available samples of this species. The tree visualizes differentiation between the two deep lineages (HE and LE) and various additional phylogroups in the LE lineage. As far as available, sample names include elevation for specimens from Montagne d’Ambre National Park (MANP). Numbers at nodes are Bayesian posterior probabilities. (B) Map of northern Madagascar showing collection localities. Colors correspond to those used in the tree.

2
preserving relict populations that have disappeared from other areas of Madagascar's more southerly humid forests (Raxworthy and Nussbaum, 1994).

A better understanding of mechanisms of species diversification in Madagascar requires an in-depth exploration of the phylogenetic relationships, geographic distributions, and taxonomy of its endemic biota. In recent years, a multitude of new discoveries have been made of amphibians and reptiles from the island, many of which have been described, while others only assigned preliminary candidate species status and await formal taxonomic revision (Vieites et al., 2009; Nagy...
et al., 2012; Perl et al., 2014). Although numerous new species have been described from MANP (e.g. Raxworthy and Nussbaum, 2006; Köhler et al., 2008; Glaw et al., 2009, 2012; Ratoavina et al., 2011; Rakotoarison et al., 2015, 2017), other components of the regional herpetofauna remain taxonomically unrevised and phylogeographically unstudied, preventing a comprehensive analysis of the origins of this amphibian and reptile community.

One poorly studied group of Malagasy amphibians is the genus *Mantidactylus* (Anura: Mantellidae), a species-rich clade comprising six subgenera (Glaw and Venes, 2006, 2007), including the subgenus *Ochthomanitis*, which has been particularly taxonomically challenging and requires revision. Members of this subgenus tend to be inconspicuous riparian frogs often found perched along stream banks. This subgenus contains five described species and at least seven undescribed candidate species (Rabibisoa, 2008; Vieites et al., 2009; Randrianainia et al., 2011; Poth et al., 2013; Perl et al., 2014). Within this subgenus, two species have undergone taxonomic changes: *M. femoralis* (Boulenger, 1882), and the focal species of the present study, *M. ambreensis* Mocquard, 1895. The latter was named based on a single specimen captured on Montagne d’Ambre, without any further information on the exact collection site (Mocquard, 1895) and its close resemblance to *M. lugubris* was mentioned in this description, but all records of *M. lugubris* from this massif were subsequently considered misidentifications (Guibé, 1978; Nicoll and Langrand, 1989; Raxworthy and Nussbaum, 1994). *Mantidactylus ambreensis* was long considered to be a synonym of *M. femoralis* (e.g., Guibé, 1978) but subsequently considered as a valid species, mainly because of its distinct continuous light lateral streak (vs. poorly defined and discontinuous in *M. femoralis*: Raxworthy and Nussbaum, 1994).

*Mantidactylus ambreensis* is currently known from across a moderately large geographical range in northern Madagascar, and elevations between 400 and 1150 m a.s.l. in MANP (Raxworthy and Nussbaum, 1994; Glaw and Venes, 2007; D’Cruze et al., 2008). In the present study we analyze the phylogeography of this species across its geographical distribution, with a particular emphasis on elevational variation at its type locality, Montagne d’Ambre, and provide evidence that it constitutes a species complex consisting of at least two species-level lineages. We use sequences from multiple mitochondrial genes and a large phylogenetic dataset to reconstruct the phylogeny within the *M. ambreensis* complex, and derive testable hypotheses of biogeography and lineage diversification from our molecular results.

2. Materials and methods

2.1. Sampling

Fieldwork was carried out on Montagne d’Ambre National Park, located between 12.34–12.75° S and 49.05–49.26° E (Goodman et al., 2018). From November 2017 to January 2018, a total of 17 sites were selected and sampled to cover a wide range of elevations (from ca. 600 to 1470 m a.s.l.) and different forest vegetation types within MANP. Individuals of *M. ambreensis* were opportunistically captured along streams, waterfalls, and pools during diurnal and nocturnal searches. For each captured individual the following information was noted: date, time, weather, habitat, substrate, sex, and geographic coordinates using a Global Positioning System receiver (GPSMAP™64s GARMIN) (see Table SM1 for geographical coordinates of collecting localities). Methods used for tissue sampling adhered to the standard procedure of National Wildlife Health Center, USA. Individuals were web or toe-clipped, their wounds were disinfected with a pain relieving, antiseptic and antibacterial Bactine® spray before being released at the capture site. Samples were stored in pre-labeled tubes filled with 100% ethanol. Samples for genetic comparisons also included additional localities in northern Madagascar collected in the period 2000–2016, as well as nominal and undescribed candidate species within the subgenus *Ochthomanitis*, and material of *M. argenteus* (subgenus *Maitsomantis*) used as outgroup. For a list of all samples used, see Supplementary Table SM1. Acronyms included FGZC, FGMV, ZCMV, and DRV referring to field numbers of F. Glaw, M. Vences, and D. R. Vieites; MSZC and MSTIS to field and tissue numbers of M. D. Scherz; and SRTIS to field numbers of S. M. Rasolonjatovo.

2.2. DNA extraction, PCR and Sanger sequencing

Total DNA was extracted using proteinase K digestion, followed by a standard salt extraction protocol (Bfrudorf et al., 1992). The reconstruction of phylogeographic relationships was based on DNA sequences of five mitochondrial genes: 12S rRNA (12S), 16S rRNA (16S), Cytochrome c oxidase subunit I (COI), Cytochrome b (COB), and NADH dehydrogenase subunit 1 (ND1). Sequences were also obtained for one nuclear gene, the recombinase activating gene 1 (RAG1), analyzed separately to obtain evidence for genealogical concordance between mitochondrial and nuclear markers. Stretches of the target genes were amplified following standard cycling protocols of polymerase chain reactions (PCR), with primers and their sequences given in Supplementary Materials Table SM2. Total PCR reaction volume contained standardized amounts of 2.5 µl 5X colorless GoTag reaction buffer, 0.1 µl GoTag DNA polymerase (5 U/µl), 0.25 µl dNTPs (10 mM), 0.3 µl of each primer (10 pmol), 8.05 µl of MQ water, and 1 µl of DNA. Amplified products were purified and directly sequenced on an automated ABI 3130xl DNA capillary sequencer. Chromatograms of the newly determined sequences were quality controlled and manually edited when necessary using CodonCode Aligner v. 3.5.6 (CodonCode Corporation). Unreliable sequences were excluded. Heterozygote positions for sequences of RAG1 were identified using the same program. For sequence alignment, we used MEGA 7 (Kumar et al., 2016). The newly generated sequences (all submitted to GenBank; accession numbers MN628333–MN628413, MN628983–MN628984, MN628991–MN628999, MN634251–MN634340) were combined with those from previous studies (Randrianainia et al., 2011; Poth et al., 2013).

2.3. Probe design for sequence capture

The sequence capture probe set used for this study is the FrogCap Ranoidea v2 probe set (Hutter et al., 2019; available at: https://github.com/chatter/FrogCap-Sequence-Capture). Probe design is described in this preprint (Hutter et al., 2019), in detail, and is summarized here. Probes were synthesized as biotinylated RNA oligos in a myBaits kit (Arbor Biosciences, formerly MYcroarray Ann Arbor, MI) by matching 25 publicly available transcriptomes to the *Nanorana parkeri* and *Xenopus tropicalis* genomes using the program BLAT (Kent, 2002). Matching sequences were clustered by their genomic coordinates to detect presence/absence across species and to achieve full marker coverage. To narrow the marker selection to coding regions, each cluster was matched to available coding region annotations from the *Nanorana* genome using the program BLAT (Sun et al., 2015). Markers from all matching species were then aligned using MAFFT (Katoh and Standley, 2013) and subsequently separated into 120 bp-long bait sequences with 2x tiling (50% overlap among baits) using the myBaits-2 kit (40,040 baits) with 120mer sized baits. These markers have an additional bait at each end extending into the intronic region to increase the coverage and capture success of these areas. Baits were then filtered, retaining those without sequence repeats, a GC content of 30–50%, and baits that did not match to multiple places in the genome. Additionally, 2300 ultra-conserved elements (UCEs) and 95 commonly used Sanger-based legacy markers from phylogenetic analyses of frogs (i.e. RAG1, POMC, TYR; Feng et al., 2017) were included to enable direct comparisons and inclusion of publicly available data from past phylogenetic studies.
2.4. Sequence capture library preparation, sequencing and data processing

We selected five samples from the ingroup focal species, two outgroups from *Mantidactylus* and one from *Boophis*. Genomic DNA was extracted from these eight tissue samples using a PROMEGA Maxwell bead extraction robot. The resultant DNA was quantified using a Quantus DNA Broad-range assay (PROMEGA). Approximately 1000 ng total DNA was acquired and set to a volume of 50 µl through dilution (with H₂O) or concentration (using a vacuum centrifuge) of the extraction when necessary.

The genomic libraries for the samples were prepared by Arbor Biosciences library preparation service (Ann Arbor, Michigan, USA). Prior to library preparation, the genomic DNA samples were quantified with fluorescence and up to 4 µg was then taken to sonication with a QSonica Q800R instrument. After sonication and SPRI bead-based size-selection to modal lengths of roughly 300 nt, up to 500 ng of each sheared DNA sample were taken to Illumina TruSeq-style sticky-end library preparation. Following adapter ligation and fill-in, each library was amplified for six cycles using unique combinations of i7 and i5 indexing primers, and then quantified with fluorescence. For each capture reaction, 125 ng of eight libraries were pooled, and subsequently enriched for targets using the MYbaits v 3.1 protocol. Following enrichment, library pools were amplified for 10 cycles using universal primers and subsequently pooled in equimolar amounts for sequencing. Samples were sequenced on an Illumina HiSeq X Ten with 150 bp paired-end reads.

A bioinformatics pipeline for filtering adapter contamination, assembling markers, and exporting alignments (Hutter et al., 2019). The pipeline is scripted in R statistical software (R Development Core Team, 2018) using the BIOCONDUCTOR suite of packages (Ramos et al., 2017) in addition to open source software publicly available and commonly used in bioinformatics. First, the raw reads were cleaned of adapter contamination, low complexity sequences, and other sequencing artifacts using the program FASTP (default settings; Chen et al., 2018). Adapter-cleaned reads were then matched to a database of bacterial and other genomes to ensure that no contamination was present in the final dataset. We decontaminated the adapter-cleaned reads with the program BBMap (https://jgi.doe.gov/data-and-tools/bbtools/), where we matched the cleaned reads to each contamination genome at > 95% similarity. Next, we merged paired-end reads using BBMerge (Bushnell et al., 2017) and removed duplicates using “de-dupe”. The merged singletons and paired-end reads were next *de novo* assembled using the program SPADES v.3.12 (Bankevich et al., 2012), which runs BAYESHAMMER (Nikolenko et al., 2013) error correction on the reads internally. Data were assembled using several different k-mer values (21, 33, 55, 77, 99, 127), where orthologous contigs resulting from the different k-mer assemblies were merged.

2.5. Sequence capture probe matching and alignment

The consensus haplotype contigs were then matched against reference marker sequences from the *Nanoranaarkeri* genome used to design the probes with BLAST (dc-megablast), keeping only those contigs that matched uniquely to the reference probe sequences. Contigs were discarded if they did not match to at least 30% of the reference marker. Finally, we merged all discrete contigs that matched to the same reference marker, joining them together with Ns based on their match position within the marker. The final set of matching contigs was named based on the marker followed by the sample name in a single file to be parsed out separately for multiple sequence alignment.

The final set of matching contigs was next aligned on a marker-by-marker basis using MAFFT local pair alignment (max iterations = 1000; ep = 0.123; op = 3). We screened each alignment for samples that were greater than 40% divergent from the consensus sequence, which are almost always incorrectly assigned contigs. Alignments were kept if they had greater than 3 taxa, had more than 100 base pairs, and a mean breadth of coverage (i.e. number of bases per sample/total alignment length) of greater than 50% across the alignment. We applied internal trimming using TRIMAL (automatic1 function; Capella-Gutiérrez et al., 2009). All alignments were externally trimmed to ensure that at least 50% of the samples had sequence data present. Finally, to minimize missing data, we retained markers when the number of samples in each alignment was greater than 75% (i.e. 6/8 of the samples). The alignment processing and filtration steps resulted in a final set of 5065 alignments. Alignments are available on Figshare (https://doi.org/10.6084/m9.figshare.10263134) and the Open Science Framework (osf.io/8amh). Raw reads can be found on the NCBI SRA: BioProject: PRJNA588101; BioSamples: SAMN13226893-SAMN13226900.

2.6. Phylogenetic analyses

We assembled four datasets for separate analysis:

(1) To obtain a first understanding of mitochondrial differentiation among all available samples of *Mantidactylusambreensis*, we first analyzed 16S sequences (520 bp) of 80 individuals, with *M. sp. Ca42* as the outgroup. We determined a general time-reversible GTR + I + G model as best-fitting this data set based on the Bayesian Information Criterion implemented in MEGA7 (Kumar et al., 2016). Bayesian inference analyses of the 16S gene dataset was executed in MrBayes v. 3.2 (Ronquist et al., 2012), implementing 10 million generations. Chains were sampled every 100th generation with a random starting tree, and with a default burn-in of 25% of the trees after assessing MCMC convergence. Genetic divergences for the 16S gene were calculated as uncorrected pairwise distances (p-distances) in MEGA7.

(2) Sequences of the nuclear gene RAG1 (299 bp for 77 individuals of *M. ambreensis*) were analyzed separately to obtain evidence from unlinked loci (mitochondrial vs. nuclear) for genetic differentiation of lineages. Alleles of the RAG1 fragment were inferred using the PHASE algorithm (Stephens et al., 2001) implemented in DnaSP software v. 5.10.3 (Librado and Rozas, 2009). Due to expected low divergence, rather than performing a phylogenetic analysis we constructed haplotype (allele) networks using the software haploviewer. Written by G. B. Ewing (http://www.cibiv.at/~greg/haplovew), this software implements the methodological approach of Salzburger et al. (2011).

(3) To test monophyly and intraspecific phylogeography of the *M. ambreensis* complex from a mitochondrial perspective, sequences of multiple mitochondrial genes were concatenated for representative individuals of all major phylogroups of the *M. ambreensis* complex, and aligned with sequences of all four described species and seven candidate species in the subgenus *Ochthomantis*. After determining the best scoring partitioning scheme and appropriate substitution models with PartitionFinder v. 2.1.1 (Lanfear et al., 2017), the concatenated alignment was subject to BI searches in MrBayes. Table SM3 gives the substitution models and partitions applied for the multigene phylogenetic reconstruction. Partitions were defined for all protein-coding genes: per single gene and by treating the 1st, 2nd, and 3rd codon position separately. We ran the analysis for 50 million generations, and chains were sampled every 1000 generations. A relative burn-in of 50% of the samples were discarded after assessing MCMC convergence.

(4) To estimate phylogeographic trends from concatenated phylogenomic (FrogCap sequence capture) data, we used the maximum-likelihood method implemented in IQ-Tree v.1.6.7 (Nguyen et al., 2015). For these analyses, we initially partitioned the data matrix by marker and tested for models of molecular evolution identified via ModelFinder (Kalyaanamoorthi et al., 2017) built into IQ-Tree, which identified an optimal partitioning scheme and models of molecular evolution for each partition. We assessed support for the resulting
topology using 1000 ultrafast bootstrap replicates (Minh et al., 2013). We also tested the results of analysis separated into UCEs, exons, and introns and different levels of missing data (50% and 75%) and found all the topologies (not shown) to be identical to the one presented here (Figs. 2 and 3).

3. Results and discussion

The main goal of this study was to test for genetic differentiation of populations of our target species, Mantidactylus ambreensis, across the environmental gradients found within MANP. Indeed, we found a surprisingly strong phylogeographic structure based on 16S sequences. Our analysis recovered two deep mitochondrial clades within the Mantidactylus ambreensis complex (Fig. 1): the high elevation lineage (HE) restricted to 940–1375 m a.s.l. in MANP, and the low elevation lineage (LE) occurring at 535–1010 m a.s.l. within MANP but also elsewhere in northern Madagascar, at an overall elevational range of 285–1040 m a.s.l. Uncorrected 16S pairwise divergences between HE and LE individuals were substantial, ranging between 5.6 and 6.8%, and the two lineages were also differentiated in the nuclear RAG1 gene (Fig. 2). In the HE lineage, all samples had identical 16S sequences except 11 samples from sites > 1300 m a.s.l., which differed by 0.4% uncorrected pairwise divergence. On the contrary, the LE lineage was further separated into four well-supported phylogroups LE1–LE4, differing by 1.5–2.8%. The analysis of concatenated sequences of multiple mitochondrial genes of M. ambreensis and of several closely related species confirmed that the two main lineages, HE and LE, together form a monophyletic group (PP = 1; Fig. 3A) within the subgenus Ochthomantis. Phylogroup LE4 from MANP was sister to LE3 from Tsaratanana (PP = 1), together this clade was sister to LE2 from Manongarivo + Maromandra (PP = 1), and this clade in turn was sister to LE1 from Makira + Bivitagno + Irogno (PP = 1). Relationships identical to those of the mitochondrial tree were suggested by the phylogenomic data (75% complete sequence capture data set, 5065 alignments of nuclear markers only), with maximum (100%) bootstrap support for all nodes (Fig. 3B). Once again, lineage HE was sister to lineage LE, and within the latter, the phylogroup LE4 from MANP was deeply nested among the other phylogroups.

Consequently, both the mitochondrial and the nuclear (sequence capture) data strongly supported that M. ambreensis complex specimens from MANP lower vs. higher elevational zones lacked direct sister relationship despite their geographical proximity. These results indicate that the two lineages on Montagne d’Ambre most likely derived from two independent colonization events of the massif.

The phylogenetic relationships within M. ambreensis, congruently inferred from mtDNA and nucDNA, are suggestive of a scenario where first, a widespread ancestral species separated into two refugial populations, one in the extreme north of Madagascar and one in an area further south, the latter latitudinally at the level of the Makira region, which today marks the southernmost known occurrence of the M. ambreensis complex. These two populations diverged in allopatry into the two main lineages of the complex (HE and LE), possibly already driven by adaptation to habitats in different elevational zones. Subsequently, the southern (LE) lineage underwent a stepwise range extension, colonizing various montane areas in a northward direction, with recurrent differentiation processes that led to the current phylogroups LE1–LE3. Eventually, the species reached Montagne d’Ambre where secondary contact with the northern (HE) lineage was established. Whether the HE population already was specialized to habitats > 900 m a.s.l., or was pushed to this elevational zone by competition with the new LE colonizers, remains unsolved.

In the haplotype network reconstruction from sequences of the nuclear RAG1 gene (Fig. 2), HE and LE showed no allele sharing at MANP. The concordant signal between the two unlinked markers, 16S and RAG1, indicates that gene flow between the two lineages is extremely limited, despite no obvious physical barrier separating them. This suggests species-level divergence under the genealogical concordance species criterion (Avise and Ball, 1990; Avise and Wollenberg, 1997). An extensive morphological analysis or DNA sequences from the type specimens will be necessary before the name M. ambreensis can be confidently applied to one of these lineages.

The contact zone of these two species-level lineages (HE and LE) currently subsumed under the name M. ambreensis was observed at an intermediate elevation (900 m a.s.l.) in MANP, close to the transition to medium altitude moist evergreen forest (Goodman et al., 2018) and coinciding with pronounced herpetological species turnover on the massif (Raxworthy and Nussbaum, 1994). Besides the M. ambreensis complex, various examples of closely related species differing in elevational distribution were reported from this massif (e.g., Rakotoarison et al., 2015, 2017). There are other cases of sympatric sister species of anurans in Madagascar; the best documented examples are the morphologically cryptic but bioacoustically differentiated such as Boophis narinsi and B. majori in the Ranomafana area (Vences et al., 2012), or the recently described Mantidactylus schultzi and its still undescribed sister species from the Tsaratanana and Manongarivo Massifs (Vences et al., 2018). Such occurrences of closely-related cryptic species highlight the role of adaptive divergence in generating the current species diversity of Madagascar (Brown et al., 2016). However, recent studies have shown that congeneric species occurring in the same mountain range may not form a monophyletic group, such as the species assemblage of Stumpflia occurring on the Marojejy Massif (Rakotoarison et al., 2019), or the elevationally separated species of Cophyla occurring on Montagne d’Ambre (Rakotoarison et al., 2015). The example of M. ambreensis demonstrates that the underlying scenarios can be notably complex, and sequential combinations of allopatric separation and adaptive divergence may in fact be the rule rather the exception.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.106700.

References

riparian Mantidactylus (Brygoomantis) frog from the Tsaratana and Manongarivo Massifs in northern Madagascar. Zootaxa 4486, 575–588.