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First amphibian inventory of one of Madagascar's smallest protected areas, Ankafobe, extends the range of the Critically Endangered *Anilany helenae* (Vallan, 2000) and the Endangered *Boophis andrangoloaka* (Ahl, 1928)

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Abstract. Using DNA barcoding, we present the first inventory of amphibians present in one of Madagascar's smallest protected areas, Ankafobe. This small area represents some of the last remaining central high plateau forests in Madagascar. However, with just 27.89 ha of forest split into two fragments, Ankafobe is highly threatened by yearly grassland fires. We recorded a total of 382 individuals and identified 14 species of frogs. This list includes the Critically Endangered *Anilany helenae* which was previously thought to be micro-endemic and restricted to Ambohitantely Special Reserve, therefore increasing this tiny species' range to a second protected area. Our results also imply an extension of the known range of the Endangered *Boophis andrangoloaka*. While our inventory likely underestimates the diversity of frog species present in Ankafobe, it provides a baseline for future conservation efforts at this site.

Keywords. DNA barcoding, Herpetofauna, central plateau, fragmentation, range extension

Introduction

Madagascar is one of the world's biodiversity hotspots for conservation priority (Myers et al., 2000) and is home to a staggering 500+ endemic amphibian species (Perl et al., 2014). From 1953-2014, however, Madagascar lost an estimated 44% of its forest cover, with much of that now in remnant fragments, and nearly half (46%) less than 100 m from the forest edge (Vieilledent et al., 2018). It is estimated that Madagascar has already lost 42% of its biodiversity at the hand of deforestation (Allnutt et al., 2008), and the increasing loss of this key amphibian habitat puts these imperilled species at further risk of extinction. Despite this significant loss, many places still are un-surveyed, resulting in incomplete inventories of the island's flora and fauna. For example, for 130 species of Malagasy amphibians, there are only one or two reliable records, while most of the other amphibian species have fewer than ten records (Vieites et al., 2008). These figures highlight the need for amphibian inventories across the island to provide starting points for conservation practitioners to build knowledge and develop conservation strategies. This baseline knowledge can be developed to gain abundance and density estimates for species, from which conservation measures can be put in place, and these values used to assess conservation successes. Further, basic information from species inventories can contribute to ongoing research in disentangling the biogeography of Malagasy amphibians. Madagascar presents high levels of amphibian microendemism (Wollenberg et al., 2008; Vences et al., 2009, 2010; Brown et al., 2016), and novel inventories will further help understand whether for some species their small range is the result of habitat loss, or simply understudied locations as opposed to true microendemism.

Madagascar's central plateau forest is highly degraded; savannah-like grasslands dominate the landscape with some small patches of degraded forest persisting in the steep valleys along streams. It is increasingly thought

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that grasslands are a part of the highland's natural landscape (Vorontsova et al., 2016; Solofondranohatra et al., 2020), however it is in living memory that many of the valleys in this area were forested, and that these have been degraded or lost in recent years (A. Ando, pers. comms). Much of this degradation has resulted from a series of human actions: firstly due to exploitative selective logging for large trees, secondly burning for charcoal production, and finally by man-made fires that are carried by the wind across the grasslands in the dry season (Miandrimanana et al., 2019). Ambohitantely Special Reserve is one of the largest last remaining forest blocks on the central plateau, home to less than 1630 hectares of forest when all fragments are combined (ESRI World Imagery, 2018) and this area has been the focal location for researching and conserving the highland's special biodiversity (Goodman et al., 2018). Ambohitantely and its surrounding area is listed as one of Madagascar's 21 Alliance for Zero Extinction (AZE) sites (Goodman et al., 2018). Within 10 km northwest of Ambohitantely, there is a lesser-known protected area called Ankafobe, which is inconspicuously located in valleys beside the Route Nationale 4 (RN4) highway.

Ankafobe covers 133.87 ha and is one of the smallest protected areas in Madagascar. This small reserve, of which the majority is grassland, contains only 27.89 ha of forest, divided into two degraded evergreen fragments. Although isolated, Ankafobe is close to Ambohitantely and is considered to be an outlier of Ambohitantely's larger forest fragment due to its similar biodiversity (Birkinshaw et al., 2009). While these fragments are isolated and their future is uncertain, they provide evidence of the natural forest habitat which once covered more of the central highlands than at present, prior to recent anthropogenic activities (Vieilledent et al., 2018). Despite their small size, these fragments are of high conservation significance (Miandrimanana et al., 2019). Research has identified three species of lemurs inhabiting Ankafobe, one of which is threatened with extinction (the Vulnerable Goodman's Mouse lemur *Microcebus lehilahytsara* Roos & Kappeler, 2005) (Rainforest Trust, 2020), and a Critically Endangered endemic tree *Schizolaena tampoketsana* Lowry et al. 1999 (Lucas, 2014). Small 0.1 ha forest plots have revealed 87 species of trees (Miandrimanana et al., 2019), and 112 species of plants are known from the site (C. Birkinshaw, pers. comm.). Despite being understudied and having an incomplete biological inventory, this limited research from Ankafobe highlights just how important these small forest fragments are for biodiversity conservation on Madagascar's central plateau.

Amphibians in Ambohitantely have been studied in the past (Vallan, 2000), but to date, there are no published inventories for amphibians in Ankafobe. To fill this knowledge gap for amphibians in Madagascar, it is critical to understand what biodiversity Ankafobe remnant forest has and create baseline information highlighting its importance for protection and for monitoring purposes. Historical herbarium specimens show that the forest in Ankafobe is too small and degraded to still host the original set of plant species found on site when the forest was more extensive (Miller et al., 2016) and hence it is assumed that these degraded fragments have already lost some of their original biodiversity. The same is likely also the case for amphibians, so we hypothesise that Ankafobe will have a similar, but reduced, amphibian fauna to that in Ambohitantely.

Materials and Methods

Study site. Ankafobe Special Reserve (Lat -18.1089, Long 47.1932, elevation 1475 m) is a small protected area of 133.87 ha, approximately 120 km north of the capital Antananarivo and 10 km from Ambohitantely, in Madagascar's sub-humid ecoregion (Fig. 1). The forest habitat present here is humid riparian forest which follows a stream in a valley between the surrounding hills. This positioning has likely protected it from the yearly fires that travel across the grasslands (Miller et al., 2016). The site has been managed by Missouri Botanical Gardens (MBG) since 2004. It has been under temporary protection from the Madagascar Government since 2018 whilst waiting for official status within the national protected area network (it is currently listed as a Nouvelle Aire Protegee (NAP) (Goodman et al., 2018)). The site is currently managed by MBG and the local community-led organisation VOI-Sohisika.

Ankafobe consists of two forest fragments with a varying landscape (Fig. 2). Fragment 1 is surrounded by grassland and the national highway RN4 curves around the fragment's northern edge. On the eastern side of the fragment, reforestation efforts have been ongoing since 2006 (C. Birkenshaw, pers. comm.). Fragment 2 is highly degraded; a fire in 2014 scorched 9 ha of forest (Missouri Botanical Gardens, 2019), which included the remaining large trees in the northwest section of the fragment. Further, the area in the centre of fragment 2 is so heavily degraded it could almost be described as two separate forest patches. This fragment is also surrounded by grassland, and some reforestation efforts

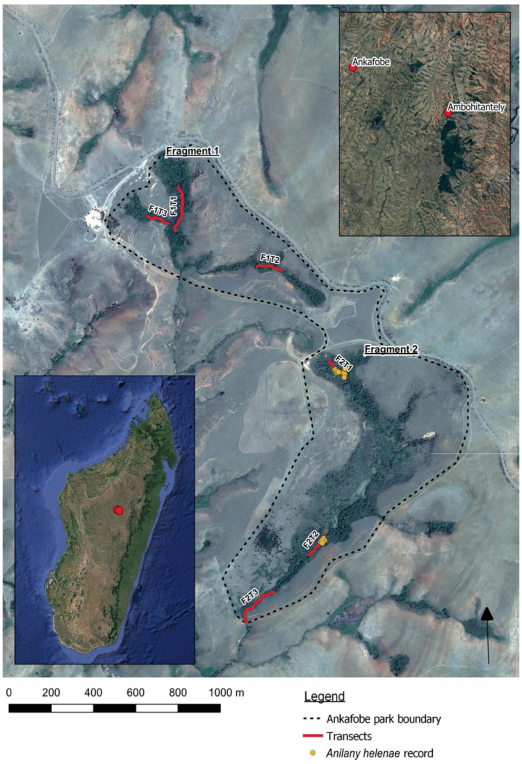

Figure 1. Map of Ankafobe protected area and location of surveyed transects in Fragments 1 and 2. Insets show the location of Ankafobe within Madagascar (bottom left) and the relative position of Ankafobe with respect to Ambohitantely Special Reserve (upper right).

in the west, while a rice paddy fragments it from a small patch of forest in the east.

Data collection. A rapid amphibian survey assessment was conducted in Ankafobe in March 2020. During six consecutive days, ten surveys were conducted across the two fragments. This resulted in 117 person-hours of survey, comprising 48 hours in the day and 69 hours at night. Both fragments had equal survey effort with three 200 m transects and a total of five surveys. Surveys were undertaken as visual encounter search surveys (VES), a method which has been proven to be the best for tropical frog sampling (Almeida-Gomes et al., 2016) and which is used to determine species richness and to compile a species list of an area (Crump and Scott, 1994). Each VES survey lasted 3 hours and was conducted along a 200 m transect (Table 1, Fig. 1). Transects were conducted to allow stratified sampling across the different microhabitats (such as riparian, swamp and terrestrial) and biotypes present in the fragments (tree trunks, tree holes, overhanging branches, forest floor leaf litter, *Pandanus* spp. leaf axils, *Dypsis* spp. axils, on and under rocks in streams, puddles, bamboo hollows, the streambeds, rock crevices, under and inside logs and in epiphytes). Quadrats (4 m^2) were also conducted randomly along the transects to target leaf litter species (Bell et al., 2006). Methods followed the 'intermediate intensity' of VES, returning all objects to their original position, reducing impact on the environment, and not destroying any features e.g., epiphytes or logs (Crump and Scott, 1994). The limitations of this were accepted to not further negatively affect an already degraded habitat. Where species were heard calling but not seen, their call was recorded, and their presence was noted. The taxonomic nomenclature used herein follows Vieites et al. (2009).

DNA barcoding. Species were identified in the field when possible, however up to three post-metamorphic individuals of each species per fragment were swabbed. Buccal swabbing allowed at least one representative of each species to be barcoded to ensure field identification was correct and to determine whether there were any cryptic and/or candidate species present. Fine tip rayon swabs (Medical Wire & Equipment Co. #MW113) were used to remove cells from inside of the frog's mouth as per the method described by Pidancier et al. (2003). The swabs were cut off into 1.5 mL Eppendorf tubes containing 0.5 - 0.75 mL of Longmire Buffer (Longmire et al., 1997).

Table 1. Coordinates (latitude and longitude) for all transects surveyed in Ankafobe.

	Fragment 1			Fragment 2		
	Transect 1	**Transect 2**	**Transect 3**	**Transect 1**	**Transect 2**	**Transect 3**
Start Lat	-18.103167	-18.106676	-18.104713	-18.110561	-18.117977	-18.121701
Start Long	47.187307	47.191886	47.186809	47.194076	47.193839	47.190324
Finish Lat	-18.10501	-18.106538	-18.104403	-18.11132	-18.118831	-18.120417
Finish Long	47.187169	47.190845	47.185937	47.194762	47.19316	47.191556

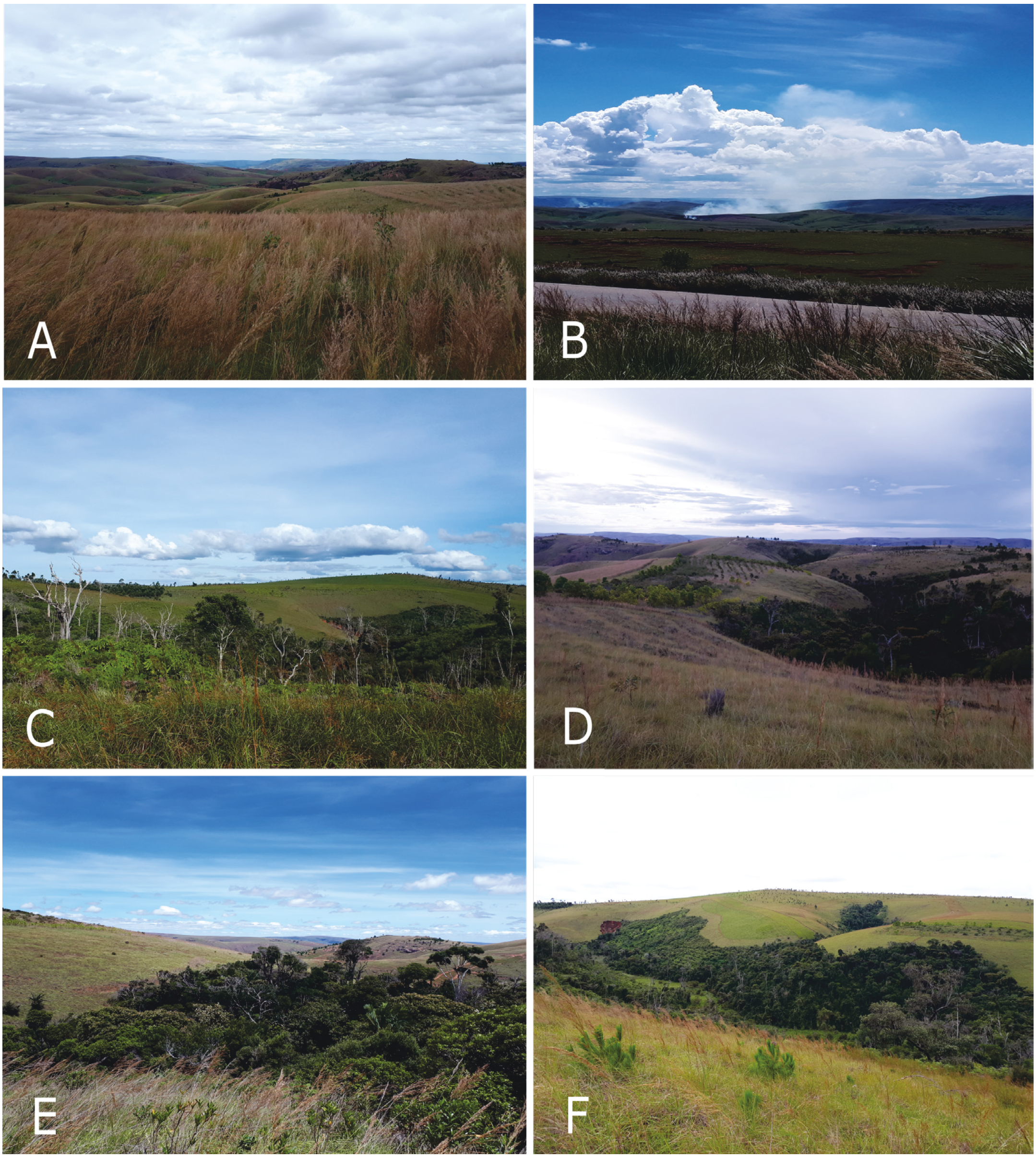

Figure 2. The landscape across Ankafobe protected area. (A) Grassland landscape (B) Man-made fires in the near distance (C) Burnt forest at the northwest side of Fragment 2 from fires in 2014 (D) East of Fragment 1 with views of reforestation efforts (E) Northeast of Fragment 1 (F) West of Fragment 2. Photographs by K. E. Mullin.

All DNA extractions were performed using the Qiagen Tissue and Blood kit following the manufacturer guidelines. Given the universal success of 16S primers and the widespread use of this mitochondrial region for amphibian barcoding (Vences et al., 2005; Vieites et al., 2009), we chose to amplify this region, sequencing at least two individual representatives from each morphologically identified species (when possible). PCR thermo-cycling conditions were as follows: 3 minutes at 94 °C, followed by 35 cycles of 94 °C for 30 seconds, 58 °C for 30 seconds and 72 °C for 1 minute, finishing with an elongation step of 72 °C for 10 minutes. A 12.5 µl reaction volume was used, using the primer pair 16SA-L, 5' - CGC CTG TTT ATC AAA

AAC AT - 3' and 16SB-H, 5' - CCG GTC TGA ACT CAG ATC ACG T - 3' (Palumbi et al., 1991). For three species identified as *Mantidactylus biporus* (Boulenger, 1889), *Mantidactylus curtus* (Boulenger, 1882), and *Spinomantis peraccae* (Boulenger, 1896), cytochrome oxidase subunit1 (COX1) was also sequenced to improve taxonomic identification. Un-purified PCR products were sequenced in the forward and reverse directions using the Eurofins sequencing service. All sequences were viewed, trimmed and aligned in Geneious Prime (https://www.geneious.com) and were run through nucleotide BLAST (NCBI) to confirm species identity. All sequences were deposited in GenBank (Table 2).

While the occurrence of *Batrachochytrium dendrobatidis* has not been previously investigated in the surveyed sites, biosecurity was taken seriously with all processing equipment sterilised between individual frogs (dipped in 25% commercial bleach, followed by 70% ethanol and then flamed) (Kolby et al., 2015). Field boots were scrubbed and disinfected with 10% commercial bleach between each fragment and field site.

Data analysis. We used species accumulation curves to indicate whether all species were likely found during the surveys, followed by species pool analysis to determine how many species were likely unseen, giving the expected total richness in the study area. These analyses were performed using the Vegan package (Oksanen, 2017) for R language (R Core Team, 2020) using specaccum (Ugland et al. 2003) for the accumulation curve and the specpool and estimateR functions for the species pool analysis. Shannon Index values were calculated to determine site species richness, and these were also computed in R using the Vegan package.

Results and Discussion

Species richness at Ankafobe. Together with visual identification (and one acoustic record), DNA barcoding revealed a total of 14 species from eight genera, distributed into three families (Table 2; Fig. 3). We recorded a total of 382 individuals over the studied period, distributed across the surveyed transects (Table 3). The most species-rich family was Mantellidae, with 11 species recorded, representing 83.8% of the total number of records. Currently, with 233 species (Frost, 2021), the Mantellidae is endemic to Madagascar and Comoros and is considered the most diverse frog family in the country (Glaw and Vences, 2007) – estimated species richness for this family is 266 species (Crottini et al., 2012) out of the 533 total number of frog species estimated for Madagascar (Perl et al., 2014). Within

Table 2. List of post-metamorphic amphibians recorded in Ankafobe forest in March 2020 with GenBank accession numbers for the 16S rRNA gene fragment and COX1 gene fragment for the three candidate species.

Family	Genus	Species	Number individuals found	IUCN Status	16S GenBank Accession numbers	COI GenBank Accession numbers
Microhylidae	*Anilany*	*helenae* (Vallan, 2000)	29	CR	MW561474, MW561476	
	Platypelis	*pollicaris* (Boulenger, 1888)	29	LC	MW561460, MW561465, MW561479, MW561480	
Mantellidae	*Boophis*	*ankaratra* Andreone, 1993	1	LC	MW561486	
	Boophis	*goudotii* Tschudi, 1838	54	LC	MW561461, MW561463, MW561471, MW561484	
	Boophis	*andrangoloaka* (Ahl, 1928)	1	EN	MW561490	
	Boophis	sp. aff. *elenae* (*B. leutus* group)	2 (Acoustic)	NT	NA	
	Guibemantis	*liber* (Peracca, 1893)	42	LC	MW561455, MW561462, MW561473, MW561481	
	Mantidactylus	*betsileanus* (Boulenger, 1882)	100	LC	MW561457, MW561467, MW561482, MW561489	
	Mantidactylus	aff. *biporus* (*M.* sp. Ca17)	24	LC	MW561456, MW561469, MW561470, MW561485	MW560836
	Mantidactylus	aff. *curtus* (*M.* sp. Ca18)	58	-	MW561459, MW561468, MW561472, MW561483	MW560838
	Mantidactylus	*femoralis* (Boulenger, 1882)	35	LC	MW561464, MW561466, MW561475, MW561477	
	Blommersia	*blommersae* (Guibé, 1975)	2	LC	MW561487	
	Spinomantis	aff. *peraccae* (*S.* sp. Ca11)	1	LC	MW561478	MW560837
Ptychadenidae	*Ptychadena*	*mascareniensis* (Duméril and Bibron, 1841)	4	LC	MW561458, MW561488	

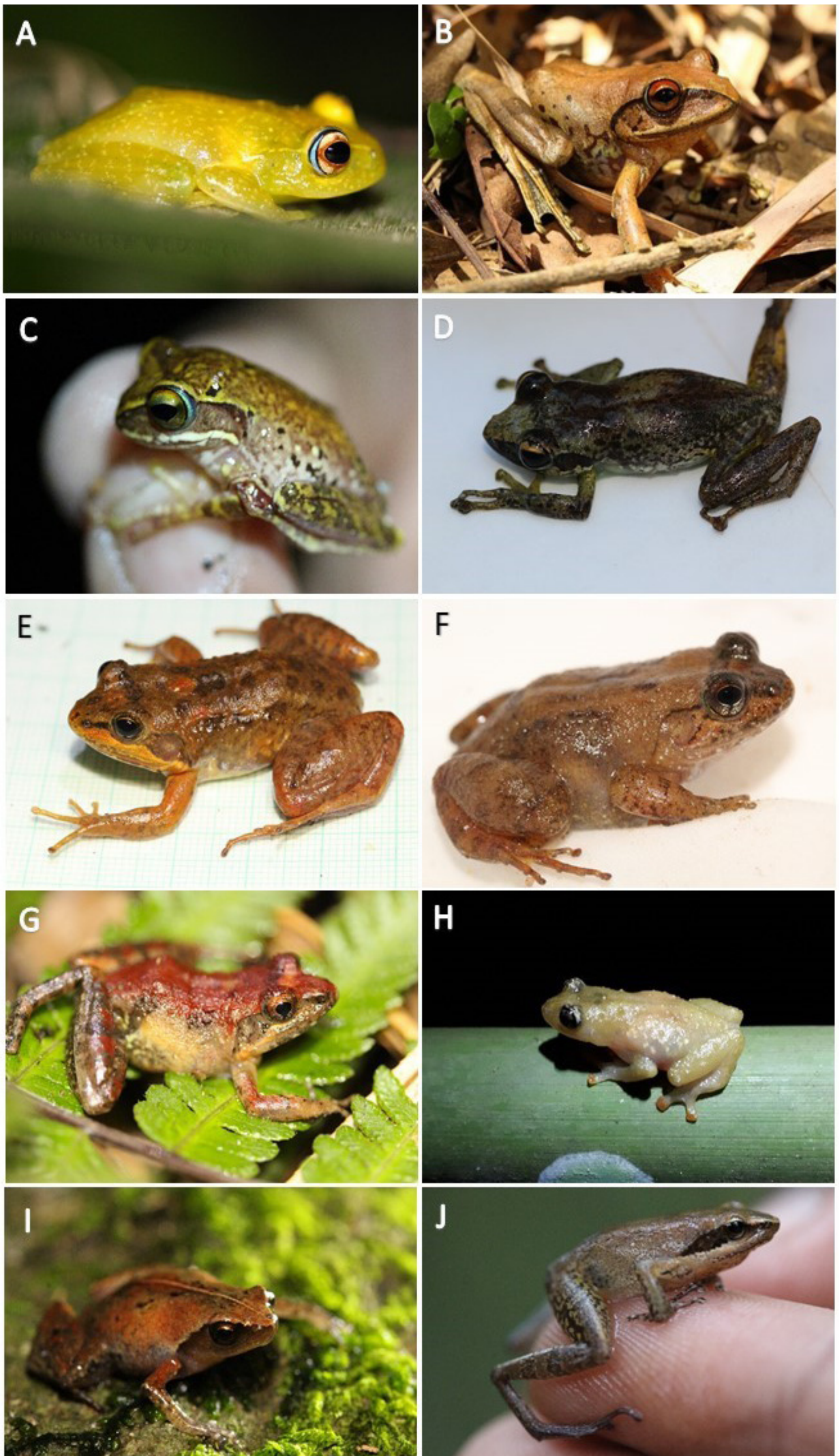

Figure 3. Ten of the 14 amphibian species found at Ankafobe during this survey: A) *Boophis ankaratra*, B) *B. goudotii*, C) *B. andrangoloaka,* D) *Guibemantis liber,* E) *Mantidactylus* sp. Ca18, F) *Mantidactylus* sp. Ca17*,* G) *Mantidactylus betsileanus,* H) *Platypelis pollicaris* I) *Anilany helenae* J) *Blommersia blommersae.* Photographs by K. E. Mullin and M.G. Rakotomanga.

Table 3. Number of individuals of each species found along each transect.

	Fragment 1			Fragment 2		
Species	**Transect 1**	**Transect 2**	**Transect 3**	**Transect 1**	**Transect 2**	**Transect 3**
A. helenae	-	-	-	19	10	-
P. pollicaris	7	4	7	1	9	1
B. ankaratra	-	1	-	-	-	-
B. goudotii	16	15	8	2	8	5
B. andrangoloaka	-	-	-	-	-	1
B. sp. aff. *elenae*	-	-	2	-	-	-
G. liber	17	1	3	10	10	1
M. betsileanus	15	27	14	11	15	18
M. aff. *biporus (M.* sp. Ca17)	1	7	4	12		
M. aff. *curtus* (*M.* sp. Ca18)	2	37	3	2	8	6
M. femoralis	5	8	18	2	1	1
Bl. blommersae	-	-	-	-	2	-
S. aff. *peraccae* (*S.* sp. Ca11)	-	-	-	1	-	-
Pt. mascareniensis	2	-	-	-	1	1
Grand Total	**65**	**100**	**59**	**60**	**64**	**34**

this family, the most represented genera in our study were *Mantidactylus* Boulenger 1895 (four species, 217 individuals) and *Boophis* Tschudi 1838 (four species, 58 individuals). Although there are currently 36 and 79 recognized species, respectively (Frost, 2020), the number of confirmed undescribed amphibian species far exceeds the number of currently described species for both genera (Vieites et al., 2009). These data show a vast underestimation of Madagascar's endemic fauna (Vieites et al., 2009; Crottini et al., 2012) and support the need for further species inventories in remaining forest fragments of un-surveyed sites (such as Ankafobe) in the central plateau of Madagascar and elsewhere. Three species were identified as confirmed candidate species (CCS *sensu* Vieites et al., 2009): *Mantidactylus* sp. Ca17 and *Mantidactylus* sp. Ca18 (Wollenberg et al., 2011), belonging to the *Mantidactylus biporus* and *Mantidactylus curtus* group, respectively; and *Spinomantis* sp. Ca11, a species belonging to the *Spinomantis peraccae* group (Perl et al., 2014).

Species range extension. Here we extend the geographical distribution of all species encountered; however, the most crucial finding is the range extension of two threatened amphibian species: *Anilany helenae* (Vallan, 2000) and *Boophis andrangoloaka* (Ahl, 1928). *Anilany helenae* was found in Ankafobe Fragment 2, extending this species' distribution 10 km northwest from the only previously known population. This species is Critically Endangered (IUCN, 2016) and was previously thought to be found in just one locality in Ambohitantely Special Reserve, where it has been found in two forest fragments (Vallan, 2000). Further surveys at other potential localities have not found any additional populations (Andreone et al., 2008). Combined with ongoing forest loss and insufficient protection of Ambohitantely, its small range warranted its status as CR. Despite our record extending the species' range to another protected area, we believe there is no need to reassess the IUCN Red List status given the small size of the fragment where it was found, the continued vulnerability of Ankafobe, and its isolation from the Ambohitantely population due to the inhospitable grasslands between the sites. This species was only recorded in the larger fragment, Fragment 2, however further intensive survey effort may also find the species in Fragment 1. Given this species' rarity, this record should be used to enhance forest conservation efforts at Ankafobe.

Another species of interest is the Endangered *Boophis andrangoloaka* (Ahl, 1928). Although just one individual of this species was found, this record also extends this species' range 10 km northwest from its previously known most western population. Having previously been known from two locations in central

Madagascar (Andrangoloaka and Ambohitantely Special Reserve), it is assumed however, to be found in locations between these two sites and is thought to occur in fewer than five threat-defined locations (IUCN, 2015). Nonetheless, species type locality (Andrangoloaka) has been dramatically reduced and a tiny portion of the original forest still exists (Andreone et al., 2007). In Ambohitantely, *B. andrangoloaka* has been recorded in fragmented forest habitat and has been found 1–1.5 m high in vegetation next to isolated trees in a large exposed swampy area (Glaw et al., 2010), and in edge forest habitat (K. Mullin, pers. obs.). Although our finding adds another protected area to the species' range, this new location is also threatened by forest loss, and the species is likely to remain endangered.

Several individuals from a *Boophis* species were heard calling from the canopy in Fragment 1. Advertisement call is the most reliable character for species identification since they differ remarkably among most species of the group (Glaw et al., 2001) and so we used acoustic records and identified this species as belonging to the *Boophis luteus* (Boulenger, 1882) group, sounding most like *Boophis elenae* Andreone, 1993. However, due to not physically seeing or DNA sampling these individuals, the species record is tentative, and the number of individuals is not accurate. *B. elenae* is listed as Near Threatened, with the true extent of its distribution considered unknown (IUCN 2015). However both *B. luteus* and *B. elenae* have been recorded at Ambohitantely (Vallan, 2000; Goodman et al., 2018), supporting our findings that a species conspecific with this lineage is present in Ankafobe. Further survey effort is required to confirm identity and distributional range of this taxon.

Amphibian community diversity. The species accumulation curve (Fig. 4) did not plateau after ten surveys and total species richness was estimated to be higher than the observed value (*Chao* = 23, SE = 9.25; *Chao1* = 15, SE = 1.80; *Jacknife1* = 18.5, SE = 2.01; *Jacknife2* = 22.5; *Bootstrap* = 15.78, SE = 0.97 and *ACE* 17.07, SE = 1.74). These values (ranging from 15 to 23) suggest that we found 60–93.3% of the species potentially present in Ankafobe. However, when a rarefaction curve is built based on the number of individuals found, rather than the number of surveys conducted, it is starting to plateau around 14 species, suggesting that not many more species than the number found should be present. Any species not surveyed may have been missed due to the lack of rains and non-destructive survey methods used (potentially missing species inside epiphytes, rotting wood, deep in the leaf litter or inside trees and bamboo). Species we expected to find include: *Spinomantis aglavei* (Methuen and Hewitt, 1913), *Boophis madagascariensis* (Peters, 1874), *Mantidactylus opiparis* (Peracca, 1893) (or other *Chonomantis* species), among others.

Despite these surveys being undertaken within the wet season, the forest leaf litter was generally very dry, with the last rains being three days before surveys began, and no rain throughout the surveys until the final one. This potentially limited the results of our rapid assessment. Across Madagascar amphibian activity is increased during the wet/warm season (November – February) (Heinermann et al., 2015), therefore further surveys should be conducted during wet months to capture explosive breeders, calling males, and more leaf litter dwelling species.

Species richness compared to Ambohitantely. The only published inventory of Ambohitantely is taxonomically out of date, however preliminary results from our research, following the same survey methodology, allow diversity comparisons between the sites. Ankafobe has a Shannon index of H = 2.09,

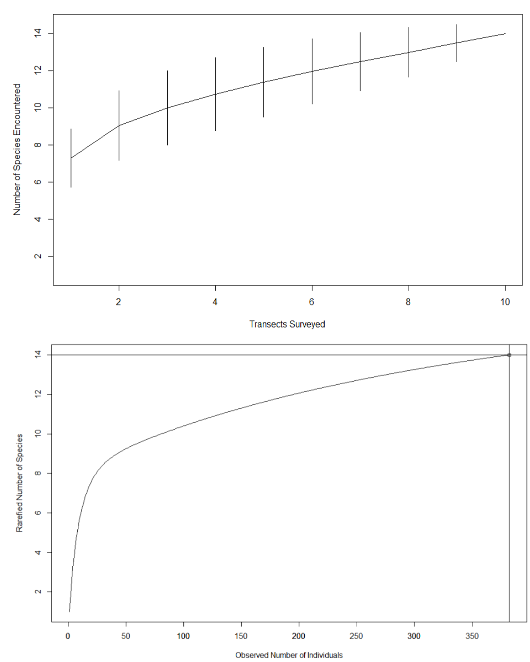

Figure 4. Species Accumulation Curve (top) based on the number of species encountered against survey effort (number of transects surveyed); vertical lines represent default confidence intervals. Rarefaction Curve of species encountered with individuals surveyed (bottom).

compared to Ambohitantely with a value of H = 2.98. As previously hypothesised, species richness is indeed lower in Ankafobe when compared to Ambohitantely. Several factors may explain this lower level of diversity.

Firstly, this reduced diversity in Ankafobe may be due to the forest being highly degraded. On average, the fragments are between 50–70 m wide (the widest forest in Ankafobe is 131 m) and are likely classified as edge habitat. Forest edges present altered microclimates and are more vulnerable to climatic influences such as solar radiation and wind, and have reduced tree cover (Dantas et al., 2016). These factors make the forest hotter and drier (Saunders et al., 1991; Alignier and Deconchat, 2013), with edge effects generally penetrating up to 100 m into the forest; however, these can vary between 5 m to 1000 m depending on location and effect being studied (Broadbent et al., 2008). Secondly, Ankafobe forest fragments may have reduced vegetation structure and structural heterogeneity, which are important factors for maintaining amphibian diversity (Vallan, 2000; Bickford et al., 2010; Ndriantsoa et al., 2017). Habitat alterations have been found to change the functional trait composition of frog assemblages in Madagascar (Riemann et al., 2017). For example, the studied fragments had very few tree holes (breeding habitats for some species) in comparison to neighbouring Ambohitantely (K. Mullin, pers. obs.).

In 2002, Ankafobe was commercially exploited for timber (Birkinshaw et al., 2009) resulting in the removal of mature trees with amphibian microhabitats (e.g. tree holes, depressions, epiphytes). This may explain why very few tree frogs were found. Other than *B. goudotii,* which was abundant, just one individual of *Spinomantis* aff. *peraccae, B. ankaratra* and *B. andrangoloaka* were recorded. Further, hole egg laying species such as *Plethodontohyla, Anodonthyla* or other *Platypelis* species (such as *Platypelis grandis*) which are present in Ambohitantely were not recorded in Ankafobe. Similar results were found in a study on fragmentation effects on amphibian communities in Eastern Madagascar, whereby large and medium-sized microhylids (specifically *Platypelis* and *Plethodontohyla*) were only found in continuous forest and not in forest fragments (Riemann et al., 2015). Additionally, Vallan (2000) found fragments smaller than 30 ha in Ambohitantely present no microhylids, concluding that this resulted from the climate and microclimates present in the fragments. *Anodonthyla vallani* (an Ambohitantely endemic) was not found in Ankafobe which may correlate with the lack of tree holes, and dryness of the degraded forest. Potentially, this difference in vegetation structure is due to the later protection of Ankafobe and previous selective logging. Following the exploitation in 2002 the remaining degraded vegetation was more vulnerable to fires, and in 2003 around 30% of the remaining forest in Ankafobe was indeed burned by grassland fires (Birkinshaw et al., 2009). Resource extraction was suspended in 2004 by Government decree (No 23548/2018), however, it is unlikely that sufficient tree maturation has occurred since then. As such, the forest fragments in Ankafobe are likely unable to support a similar number of species to Ambohitantely. These changes could have resulted in the lower species diversity observed in this study, including the absence of tree frogs who rely upon specific microhabitats.

Conclusions. This study has extended the range of all species surveyed, and most importantly, the ranges of the Critically Endangered *Anilany helenae* and the Endangered *Boophis andrangoloaka.* Despite the limitations presented during this rapid assessment, our study provides a baseline for managers of this small but locally important protected area, feeding into conservation plans and future monitoring of the amphibian fauna. The present inventory supports the growing evidence that small forest fragments are important for species conservation in Madagascar.

These findings should contribute towards the Government's decision to make this protected area officially part of the national protected area network. Surrounding forest remnants are informally protected by local volunteers who occasionally visit forest fragments and report any illegal deforestation to local authorities. While Ankafobe forest is protected by MBG and the local community association (VOI), made up of passionate locals, the reserve is still highly threatened by unpredictable and uncontrollable man-made fires that are carried across the landscape by the wind. Approximately 70% of the local population are members of the VOI (A. Ando, pers comm.), and membership gives access to the planted *Eucalyptus* spp. forest for firewood and the opportunity to work on any jobs that arise on the reserve, such as reforestation events. Conservation efforts must continue to support the local people in preserving this forest.

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References

Alignier, A., Deconchat, M. (2013): Patterns of forest vegetation responses to edge effect as revealed by a continuous approach. Annals of Forest Science **70**(6): 601–609.

Allnutt, T.F., Ferrier, S., Manion, G., Powell, G.V.N., Ricketts, T.H., Fisher, B.L., et al. (2008): A method for quantifying biodiversity loss and its application to a 50-year record of deforestation across Madagascar. Conservation Letters **1**(4): 173–181.

Almeida-Gomes, M., Vieira, M.V., Rocha, C.F.D., Metzger, J.P., De Coster, G. (2016): Patch size matters for amphibians in tropical fragmented landscapes. Biological Conservation **195**: 89–96.

Andreone, F., Vences, M., Glaw, F., Randrianirina, I.E. (2007): Remarkable records of amphibians and reptiles on Madagascar's central high plateau. Tropical Zoology **20**(1): 19–39.

Andreone, F., Cox, N.A, Glaw, F., Kohler, J., Rabibisoa, N.H.C., Randriamahazo, H., et al. (2008): Update of the Global Amphibian Assessment for Madagascar in light of species discoveries, nomenclature changes, and new field information. Monografia Museo Regionale di Scienze Naturali (2008): 419–436.

Bell, K.E., Donnelly, M.A., Rica, D.C. (2006): Influence of forest fragmentation on community structure of frogs and lizards in Northeastern Costa Rica. Conservation Biology **20**(6): 1750–1760.

Bickford, D., Ng, T.H., Qie, L., Kudavidanage, E.P., Bradshaw, C.J.A. (2010): Forest fragment and breeding habitat characteristics explain frog diversity and abundance in Singapore. Biotropica **42**(1): 119–125.

Birkinshaw, C., Mamisoa, A., Rasolofonirina, J.J. (2009): Survival and growth of seedlings of 19 native tree and shrub species planted in degraded forest as part of a forest restoration project in Madagascar's highlands. Madagascar Conservation and Development **4**(2): 128–131.

Broadbent, E.N., Asner, G.P., Keller, M., Knapp, D.E., Oliveira, P.J.C., Silva, J.N. (2008): Forest fragmentation and edge effects from deforestation and selective logging in the Brazilian Amazon. Biological Conservation **141**(7): 1745–1757.

Brown, J.L., Sillero, N., Glaw, F., Bora, P., Vieites, D.R., Vences, M. (2016): Spatial biodiversity patterns of Madagascar's amphibians and reptiles. PLoS ONE **11**(1): 1–26.

Crottini, A., Madsen, O., Poux, C., Strauß, A., Vieites, D.R., Vences, M. (2012): Vertebrate time-tree elucidates the biogeographic pattern of a major biotic change around the K-T boundary in Madagascar. Proceedings of the National Academy of Sciences of the United States of America **109**(14): 5358–5363.

Dantas De Paula, M., Groeneveld, J., Huth, A. (2016): The extent of edge effects in fragmented landscapes: Insights from satellite measurements of tree cover. Ecological Indicators **69**: 196–204.

Doan, T.M. (2003): Which methods are most effective for surveying rain forest herpetofauna? Society for the Study of Amphibians and Reptiles **37**(1): 72–81.

ESRI 'World Imagery' (2018). https://www.arcgis.com/home/item.html?id=10df2279f9684e4a9f6a7f08febac2a9. Accessed on 15 August 2020.

Frost, D. (2021). Amphibian Species of the World 6.0: An Online Reference. Available at: https://amphibiansoftheworld.amnh.org/. Accessed 2nd February 2021.

Glaw, F., Köhler, J., De La Riva, I., Vieites, D.R., Vences, M. (2010): Integrative taxonomy of malagasy treefrogs: Combination of molecular genetics, bioacoustics and comparative morphology reveals twelve additional species of Boophis. Zootaxa **2383**: 1–82.

Glaw, F., Vences, M., Andreone, F., Vallan, D. (2001): Revision of the *Boophis majori* group (Amphibia: Mantellidae) from Madagascar, with descriptions of five new species. Zoological Journal of the Linnean Society **133**(4): 495–529.

Goodman, S.M., Raherilalao, M.J., Wohlauser, S. (2018): The terrestrial Protected Areas of Madagascar: Their history, description and biota. Edited by S.M. Goodman, M.J. Raherilalao, S. Wohlauser. Association Vahatra, Antananarivo.

Heinermann, J., Rodríguez, A., Segev, O., Edmonds, D., Dolch, R., Vences, M. (2015): Year-round activity patterns in a hyperdiverse community of rainforest amphibians in Madagascar. Journal of Natural History **49**(33–36): 2213–2231.

IUCN SSC Amphibian Specialist Group (2016): *Rhombophryne helenae*. The IUCN Red List of Threatened Species 2016: e.T58010A84183331. https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T58010A84183331.en. Downloaded on 18 June 2020.

IUCN SSC Amphibian Specialist Group (2015): *Boophis elenae* (errata version published in 2016). The IUCN Red List of Threatened Species 2015: e.T49546174A105167056. https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T49546174A49546022.en. Downloaded on 18 June 2020.

IUCN SSC Amphibian Specialist Group (2015): *Boophis andrangoloaka*. The IUCN Red List of Threatened Species 2015: e.T49380186A49380854. https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T49380186A49380854.en. Downloaded on 18 June 2020.

Kolby, J.E., Smith, K.M., Ramirez, S.D., Rabemananjara, F., Pessier, A.P., Brunner, J.L., et al. (2015): Rapid response to evaluate the presence of amphibian chytrid fungus *(Batrachochytrium dendrobatidis)* and ranavirus in wild amphibian populations in Madagascar. PLoS ONE **10**(6): 1–21.

Longmire, J.L., Maltbie, M., Baker, R.J. (1997): Use of "Lysis Buffer" in DNA isolation and its implication for museum collections. Occassional Papers Museum of Texas Tech University (163).

Lucas, J. (2014): Demographic survey of the Sohisika (*Schizolaena tampoketsana*) at Ankafobe, Madagascar. Independent Study Project (ISP) Collection 1763.

Miandrimanana, C., Reid, J.L., Rivoharison, T., Birkinshaw, C. (2019): Plant diversity planting position and shade enhance native seedling performance in forest restoration for an endangered malagasy plant. Plant Diversity **41**(2): 118–123.

Miller, J.S., Lowry II, P.P., Aronson, J., Blackmore, S., Havens, K., Maschinski, J. (2016): Conserving biodiversity through ecological restoration; the potential contributions of botanical gardens and arboreta. Candollea **71**(1).

Ministere de l'Environnement, De l'Ecologie et des Forêts Official Document (2018): Project d'arrate Interministeriel No23548/2018 Portant mise en protection temporaire de l'aire protegee en creation denommee Ankafobe District D'Ankazobe, region Analamanga. Available at: https://www.mobot.mg/conservation/wp-content/uploads/2019/11/ARRETE-de-protection-temporaire-Ankafobe-FR.pdf. Accessed on 18 July 2020.

Missouri Botanical Gardens (2019): Are we succeeding? Monitoring statistics. Available at: https://www.mobot.mg/conservation/. Accessed on 18 June 2020.

Myers, N., Mittermeler, R.A., Mittermeler, C.G., Da Fonseca, G.A.B., Kent, J. (2000): Biodiversity hotspots for conservation priorities. Nature **403**(6772): 853–858.

Ndriantsoa, S.H., Riemann, J.C., Glos, J.S., Raminosoa, N., Ro, M. (2017): Amphibian diversity in the matrix of a fragmented landscape around Ranomafana in Madagascar depends on matrix quality. Tropical Conservation Science **10**: 1–16.

Oksanen, J. (2017): Vegan: ecological diversity. R Package Version 2.4-4, 1, p. 11. Available at: https://cran.r-project.org/package=vegan.

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G. (1991): The simple fool's guide to PCR. Ver. 2, p. 46. Honolulu: University of Hawaii.

Perl, R.G.B, Nagy, Z.T., Sonet, G., Glaw, F., Wollenberg, K.C., Vences, M. (2014): DNA barcoding Madagascar's amphibian fauna. Amphibia Reptilia **35**(2): 197–206.

Pidancier, N., Miquel, C., Miaud, C. (2003): Buccal swabs as a non-destructive tissue sampling method for DNA analysis in amphibians. Herpetological Journal **13**(4): 175–178.

Rainforest Trust (2020): Last stand for Critically Endangered trees in Madagascar. Available at: https://www.rainforesttrust.org/projects/last-stand-for-critically-endangered-trees-in-madagascar/ Accessed on 18 June 2020.

Riemann, J.C., Ndriantsoa, S.H., Raminosoa, N.R., Rödel, M.O., Glos, J. (2015): The value of forest fragments for maintaining amphibian diversity in Madagascar. Biological Conservation **191**: 707–715.

Riemann, J.C., Ndriantsoa, S.H., Rödel, M., Glos, J. (2017): Functional diversity in a fragmented landscape - Habitat alterations affect functional trait composition of frog assemblages in Madagascar. Global Ecology and Conservation **10**: 173–183.

Saunders, D.A., Hobbs, R.J., Margules, C.R. (1991): Biological consequences of ecosystem fragmentation: A Review. Conservation Biology **5**(1): 18–32.

Solofondranohatra, C.L., Vorontsova, M.S., Hempson, G.P., Hackel, J., Cable, S., Vololoniaina, J., Lehmann, C.E.R. (2020): Fire and grazing determined grasslands of central Madagascar represent ancient assemblages: Grasslands are shaped by disturbance. Proceedings of the Royal Society B: Biological Sciences **287**(1927): 20200598.

Ugland, K.I., Gray, J.S., Ellingsen, K.E. (2003): The species-accumulation curve and estimation of species richness. Journal of Animal Ecology **72**(5): 888–897.

Vallan, D. (2000): Influence of forest fragmentation on amphibian diversity in the nature reserve of Ambohitantely, highland Madagascar. Biological Conservation **96(**1): 31–43.

Vences, M., Köhler, J., Pabijan, M., Glaw, F. (2010): Two syntopic and microendemic new frogs of the genus blommersia from the east coast of Madagascar. African Journal of Herpetology **59**(2): 133–156.

Vences, M., Thomas, M., Meijden, A. Van Der, Chiari, Y., Vieites, D.R. (2005): Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. Frontiers in Zoology **2**(5): 1–12.

Vences, M., Wollenberg, K.C., Vieites, D.R., Lees, D.C. (2009): Madagascar as a model region of species diversification. Trends in Ecology and Evolution **24**(8): 456–465.

Vieilledent, G., Grinand, C., Rakotomalala, F.A., Ranaivosoa, R., Rakotoarijaona, J.R., Allnutt, T.F., Achard, F. (2018): Combining global tree cover loss data with historical national forest cover maps to look at six decades of deforestation and forest fragmentation in Madagascar. Biological Conservation **222**: 189–197.

Vieites, D.R., Wollenberg, K.C., Andreone, F., Kohler, J., Glaw, F., Vences, M. (2009): Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proceedings of the National Academy of Sciences **106**(20): 8267–8272.

Vieites, D.R., Nieto-Roman, S., Vences, M. (2008): Towards understanding the spatial pattern of amphibian diversity in Madagascar. In: A Conservation Strategy for the Amphibians of Madagascar, p 397–409.

Vorontsova, M.S., Besnard, G., Forest, F., Malakasi, P., Moat, J., Clayton, W.D. et al. (2016): Madagascar's grasses and grasslands: Anthropogenic or natural? Proceedings of the Royal Society B: Biological Sciences **283**(1823): 20152262.

Wollenberg, K.C., Vieites, D.R., Meijden, A.V.D., Glaw, F., Cannatella, D.C., Vences, M. (2008): Patterns of endemism and species richness in Malagasy cophyline frogs support a key role of mountainous areas for speciation. Evolution **62**(8): 1890–1907.

Wollenberg K.C, Vieites D.R., Glaw, F., Vences, M. (2011): Speciation in little: The role of range and body size in the diversification of Malagasy mantellid frogs. BMC Evolutionary Biology **11**(1): 217.

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