

## Combining old and new evidence to increase the known biodiversity value of the Sahamalaza Peninsula, Northwest Madagascar

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### Abstract

Prior herpetological surveys in 1996 and 2000 identified 14 species of amphibians and 32 species of reptiles from the Sahamalaza Peninsula. This work increases the total number of amphibian and reptile species known from this area to 20 and 43 respectively. To maximise our chances of species detection, survey effort covered the entire wet season and part of the dry season, and utilised a combination of opportunistic searching, transect searching, pitfall trapping, and acoustic recording. We identified species through an integrative taxonomic approach, combining morphological, bioacoustic and molecular taxonomy. Together, this enabled the detection of cryptic and seasonally inactive species that were missed in the shorter prior surveys that relied on morphological identification alone. The taxonomic identification of amphibians utilised a fragment of the mitochondrial 16S rRNA gene; taxonomic identification of reptiles utilised a fragment of the mitochondrial COI gene, and when necessary, also mitochondrial fragments of the 16S rRNA ND1, ND2, ND4 genes. All sequences were deposited in Genbank and COI sequences were also deposited in the BOLD database to foster taxonomic identification of malagasy reptiles. We report two new taxa: a species of *Boophis*, since described as *B. ankarafensis*, and a candidate new species of microhylid (genus: *Stumpffia*). We document range expansions of *Boophis tsilomaro*, *Cophyla berara*, *Blaesodactylus ambonihazo* beyond their type localities. Along with significant range expansions across a range of taxa, including *Blommersia* sp. Ca05, *Boophys brachy-*

*chir*, *Brookesia minima*, *Ebenavia inunguis*, *Geckolepis humbloti*, *Madascincus stumpffi*, *Pelomedus subrufa* and *Phelsuma kochi*. Forest in the peninsula is under extreme pressure from human exploitation. Unless unsustainable agricultural and pastoral practices encroaching on these habitats halt immediately, both forest and the species that occur there, several of which appear to be local endemics, may be irreversibly lost.

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## Introduction

Madagascar ranks amongst the richest countries in the world for the diversity of its herpetofauna, harbouring about 400 described species of non-avian reptiles (from here onward, we will use the traditional term ‘reptiles’ for species included in the Sauropsida excluding birds) and about 320 described species of amphibians (Glaw and Vences, 2007; Perl et al., 2014; AmphibiaWeb, 2017). The uniqueness of present-day Madagascan biota can be partially explained by the biogeographic isolation of the island. Indeed, much of Madagascar’s extant fauna is the result of successful colonizations around the K-T boundary at ca. 60–70 mya (Crottini et al., 2012; Samonds et al., 2012). The Madagascan herpetofauna shows remarkably high levels of endemism, with 92% of non-marine reptile species and all but one of the native amphibian species found nowhere else (Glaw and Vences, 2007).

Over the last few years, large-scale taxonomic inventories, using a combination of molecular tools, bioacoustics and morphological methods have led to a rapid increase in species descriptions and in the identification of a large number of candidate species that await description (Vieites et al., 2009; Nagy et al., 2012; Rosa et al., 2012; Perl et al., 2014). Many of the newly identified taxa are easily diagnosable, while many other species that were thought to be relatively widespread across Madagascar represent complexes of several species. This resulted in several taxonomic revisions (mostly at the genus level) and in a remarkable number of new or resurrected amphibian and reptile species [e.g. *Aglyptodactylus* (Köhler et al., 2015), *Boophis* (Glaw et al., 2010), *Blommersia* (Andreone et al., 2010), *Gephyromantis* (Vences et al., 2017), *Guibemantis* (Lehtinen et al., 2011), *Mantidactylus* (Bora et al., 2011), *Scaphiophryne* (Raselimanana et al., 2014), *Anodontyla* (Vences et al., 2010a), *Cophyla* (Rakotoarison et al., 2015), *Platypelis* (Rosa et al., 2014), *Rhombophryne* (Scherz et al., 2016); *Stumpffia* (Rakotoarison et al., 2017), *Brookesia* (Glaw et al., 2012), *Furcifer* (Florio et al., 2012), *Calumma* (Gehring et al., 2011), *Chalarodon* (Miralles et al., 2015), *Zonosaurus* (Raselimanana et al., 2006), *Madascincus* (Miralles et al., 2011), *Paracontias* (Miralles et al., 2016), *Paragehyra* (Crottini et al., 2015), *Uroplatus* (Ratsoavina et al., 2011), *Phelsuma* (Crottini et al., 2011), *Liopholidophis* (Glaw et al., 2014)].

Amphibians are experiencing an unprecedented worldwide decline, 41% of the described species are threatened with extinction (Monastersky, 2014) and

species loss is occurring at more than 200 times the average background extinction rate (Roelants et al., 2007). Many reptile species are also in decline. In a representative sample of 1500 species nearly one fifth were found to be threatened (Böhm et al., 2013). The leading causative factors are the destruction, alteration, and fragmentation of habitats (Stuart et al., 2004; Andreone et al., 2005; Sodhi et al., 2008; Irwin et al., 2010; Jenkins et al., 2014). Having lost one third of its primary forest since the 1970s, Madagascar is no exception, and it continues to lose around 8600 km<sup>2</sup> (0.5%) of primary forest per year (FAO 2015). This loss will have a tremendous impact on all unique biodiversity of Madagascar, including amphibian and reptile species due to their specific habitat requirements coupled with a high dependency on the stability and quality of their habitats (Andreone et al., 2005; Sinervo et al., 2010; Riemann et al., 2015), and most probably also on human communities.

Climatic change is likely to intensify the effects of Madagascar’s habitat loss (Raxworthy et al., 2008; Huey et al., 2009; Walls et al., 2013), as will the recent discovery of potentially emergent infectious pathogens (Bletz et al., 2015a, 2015b; Kolby et al., 2015), and the introduction of invasive species (Andreone et al., 2014; Crottini et al., 2014; Kolby et al., 2014; Vences et al., 2017).

A large proportion of Madagascar’s amphibian and reptile diversity is limited to the island’s northern and eastern rainforest slopes, which are known to host a high number of endemic species (e.g. Rosa et al., 2012; Heinermann et al., 2015; Brown et al., 2016). In recent years, high levels of species diversity have also been described from the west of the island (e.g. D’Cruze et al., 2006; Mercurio et al., 2008; Bora et al., 2010). Many reptile and amphibian species are known exclusively from western dry forests, such as several species of Gerrhosauridae (Raselimanana, 2003), Opluridae (Raselimanana et al., 2000) and tree frogs (Penny et al., 2014), most of which have narrow ranges. Dry forests, in particular those in the sub-arid regions of Madagascar, are poorly understood in terms of flora and fauna (Sussman and Rakotozafy, 1994). Malagasy deciduous dry forests declined in primary forest cover from 12.5% in 1950 to 2.8% in 1990 (Smith, 1997) and, due to their susceptibility to fire and conversion to agricultural land, are among one the most threatened habitats in the country (Janzen, 1988; Pons et al., 2003; Elmquist et al., 2007). Forest destruction was further exacerbated by a political coup in 2009, which led to a weakening in government enforcement (Schuurman

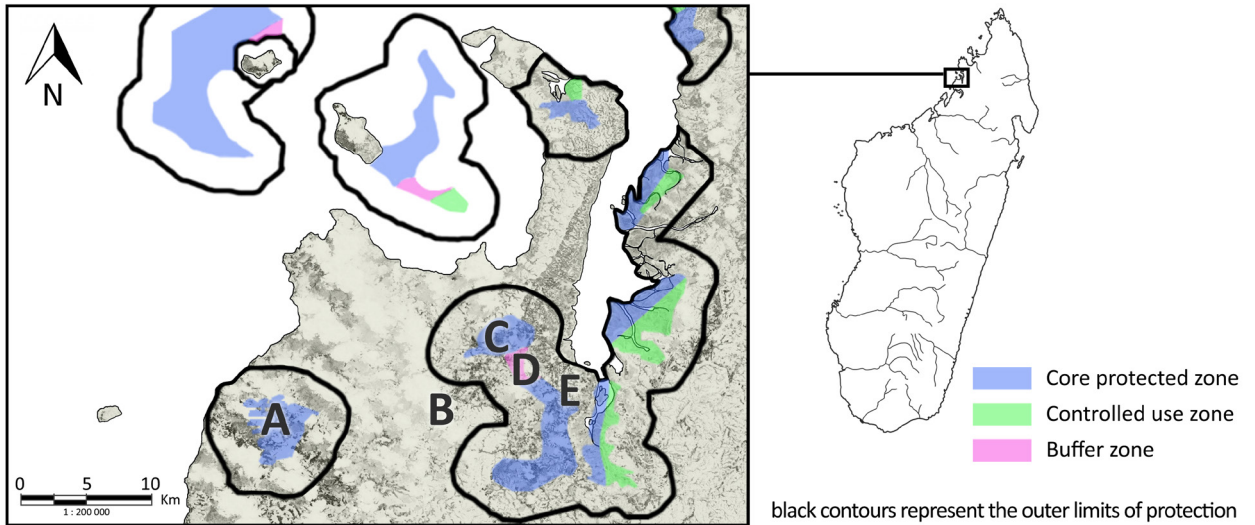


Figure 1. The Sahamalaza Peninsula in northwestern Madagascar, indicating the study sites of (A) Ankarafa Forest, (B) Antafiabe village, (C) Berara (Anabohazo Forest), (D) Anketsakely (Anabohazo Forest) and (E) Betsimipoaka village.

and Andreone, 2010; Andreone et al., 2012; Schwitzer et al., 2014). Despite acquiring formal protection in 2007, the Sahamalaza Peninsula, in western Madagascar, still experiences high levels of anthropogenic pressure on its terrestrial, freshwater and marine ecosystems (Schwitzer et al., 2007; Seiler et al., 2012; Penny et al., 2014). No large intact areas of primary forest remain, with forest consigned to a matrix of small isolated fragments, all of which show some degree of anthropogenic disturbance and/or edge effects (Schwitzer et al., 2007). The human communities living in the periphery of the protected area depend on subsistence agriculture (through ‘slash-and-burn’) and fishing for their livelihoods, which traditionally occurred in the core zones of the park.

A total of 14 species of amphibians and 32 species of reptiles were previously documented from the Sahamalaza Peninsula (Andreone et al., 2001; Raselimanana, 2008). The conservation importance of this community is high, due to the presence of several microendemic and threatened species. Species with spatially narrow niches are often more sensitive to the microhabitat changes associated with disturbance, thus it is particularly important to collect further ecological and distributional data on them (Glos et al., 2008; Irwin et al., 2010). To implement an effective conservation plan for Sahamalaza’s herpetofauna it is crucial to increase our knowledge on the distribution and ecology of the species that occur here (Penny et al., 2016), particularly for the local endemics that were

discovered before much of the recent habitat destruction had occurred. Using an integrative taxonomic approach to species identification, we here provide an update on the presence and distribution of amphibian and reptile species found on the Sahamalaza Peninsula.

## Methods

### Study site

Surveys took place in the Sahamalaza Peninsula, in the province of Mahajanga, Northwest Madagascar (Figure 1). The peninsula covers approximately 26,000 hectares and is defined by the Sahamalaza Bay to the east, the Mozambique Channel to the west and the Loza River to the south (Volampeno, 2009). Parts of the peninsula were designated a UNESCO Biosphere Reserve in 2001, followed by the creation of the Sahamalaza-Îles Radama National Park in July 2007 (Schwitzer et al., 2007).

The area has a sub-humid climate with two distinct seasons: a hotter, wetter season from December to April and a cooler, drier season from May to November. Monthly mean maximum temperature ranges from  $28.5 \pm 3.61$  °C in July to  $39.1 \pm 2.11$  °C in February; while monthly mean minimum temperature ranges from  $13.2 \pm 0.81$  °C in October to  $21.8 \pm 0.81$  °C in January (Volampeno et al., 2011). The mean

annual precipitation rate is around 1600 mm (Schwitzer et al., 2007). This climate supports a unique type of hybrid forest, consisting of plant species from both the wetter Sambirano domain and drier Western domain (Birkenshaw, 2004; Schwitzer et al., 2006). The forest consists of a matrix of small fragments isolated by savannah, all subjected to high levels of human disturbance (Schwitzer et al., 2007).

Prior herpetological survey efforts were focused on Analavory Forest (14°23.30' S, 47°56.15' E; Raselimanana, 2008), since destroyed by fire in 2004 (Volampeno, 2009), and the Berara Forest fragment in Anobohazo (14°18.6' S, 47°54.9' E; Andreone et al., 2001). The present survey revisited Anobohazo, including the fragment of Anketsakely in addition to Berara, and surveyed the Ankarafa Forest (14°22.8' S, 47°45.5' E) for the first time. The surroundings of Antafiabe (14°21.3' S, 47°52.1' E), and Betsimipoaka (14°19.8' S, 47°57.8' E) villages were also surveyed. Surveys were conducted between October 2011 and January 2012, and between January and February 2013. This ensured coverage of the entire wet season, when individuals are expected to be more active, and the end of the dry season.

#### *Survey methods*

Survey methods included opportunistic searching, transect searching, pitfall trapping and acoustic recording. Transect searches were repeated during the day and night to account for any diel differences in activity, taking place in the morning and evening. Searching took place approximately two metres either side of the transect and up to two metres in height, and for amphibians were directed towards vocalising males. Searches in Ankarafa occurred in both the dry and wet season (during the 2011 period) and followed the same routes where possible. Sites were sampled in a randomised order and all searches were conducted by the same two individuals to avoid systematic observer bias. Location was logged using a handheld GPS receiver (Garmin eTrex Vista HCx; Garmin International Inc., Olathe, USA). Representative individuals were photographed to document their coloration, using a digital camera; tissue samples were collected, as were call recordings of amphibians. An integrative taxonomic approach was taken to assess species identification of both amphibians and reptiles; utilising the keys provided by Glaw and Vences (2007, and subsequent publications), personal photographic and acoustic catalogues, the application of molecular taxonomic identification as well as the comparative

material hosted in the herpetological collection of the Museo Regionale di Scienze Naturali, Torino, Italy.

Pitfall traps with drift fences were made by sinking plastic buckets (270 mm deep, 220-250 mm internal diameter) into the ground at 6 m intervals along a 30 m drift fence, 0.4 m high, and buried 50 mm deep. Plant detritus was placed in the bottom of each bucket to act as a refuge for animals and holes punched in the bottom to allow water to drain. The pitfalls were checked each morning and evening for captured animals, and non-target animals were released. An initial four pitfall lines constructed in Ankarafa Forest in October 2011 were checked for a period of 13-15 days; these proved to be ineffective and inefficient, so a large scale expansion of pitfall trapping was discounted. A further three pitfall lines were constructed in Ankarafa Forest along a ridge, a slope and a valley bottom, for two periods of 14-15 days in October/November 2011 and December/January 2011-2012, covering the dry and wet seasons.

#### *Molecular taxonomic identification*

Tissue samples were collected with a maximum of five individuals per species-level taxon per population. If individuals appeared to belong to new and undescribed species, a limited number of voucher specimens were collected, as advised by the Code of Zoological Nomenclature (ICZN 1999). These were anaesthetised (by immersion in MS222), and fixed in 10% buffered formalin or 90% ethanol, and later transferred in 65-75% ethanol. Voucher specimens were deposited in the Museo Regionale di Scienze Naturali, Torino, Italy, the Parc Botanique et Zoologique de Tsimbazaza (PBZT), Antananarivo, Madagascar, and Mention Zoologie et Biodiversité Animale, Faculté des Sciences, Université d'Antananarivo, Madagascar (UADBA). Most of the tissue samples were collected in the 2013 expedition and only a small number of tissue samples were collected in the 2011-2012 surveys.

Total genomic DNA was extracted from the tissue samples using proteinase K digestion (10 mg/ml concentration) followed by a standard salt extraction protocol (Bruford et al., 1992). A fragment of ca. 550 bp of the 3' terminus of the mitochondrial 16S rRNA gene (16S), proven to be suitable for amphibian identification (Vences et al., 2005a), was amplified for 78 amphibian tissue samples, while a fragment of around 650 bp of the standard barcoding region of the cytochrome c oxidase subunit I gene (COI) (Nagy et al., 2012) was amplified for 42 reptile tissue samples and one amphibian (Table S1). In reptiles the molecular taxonomic

Table 1. Primer information (gene fragment, primer name, sequence, literature source) and PCR conditions used for the present study.

Gene	Primer name	Sequence (5'-3')	Source	PCR conditions
<b>16S rRNA</b>	AC_16s_ar	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)	94 (90), [94 (45), 55 (45), 72 (90) x33], 72 (600)
	AC_16s_br	CCGGTYTGAACCTCAGATCAYGT	Modified from Palumbi et al. (1991)	
<b>COI</b>	RepCOI-F	TNTTMTCAACNAACCACAAAGA	Nagy et al. (2012)	94 (180), [94 (40), 49 (30), 72 (60) x40], 72 (420)
	RepCOI-R	ACTTCTGGRTGKCCAAARAATCA	Nagy et al. (2012)	
<b>COI amphibians</b>	dgLCO1490	GGTCAACAAATCATAAAGAYATYGG	Meyer et al. (2005)	94 (90), [94 (30), 49 (45), 72 (90) x35], 72 (600)
	dgHCO2198	TAAACTTCAGGGTGACCAARAAYCA	Meyer et al. (2005)	
<b>ND1 + associated tRNAs</b>	ND1 intf2	AAYCGVGCVCCWTTYGACCTWACAGA	Schmitz et al. (2005)	95 (120), [95 (30), 50 (30), 72 (60) x40], 72 (600)
	ND1 tmet	TCGGGGTATGGGCCCRARAGCTT	Leaché and Reeder (2002)	
<b>ND2</b>	Ala-R2	AAAATRTCTGRGTTGCATTTCAG	Macey et al. (1997)	94 (90), [94 (30), 45 (45), 72 (90) x35], 72 (600)
	ND2_f17	TGACAAAAAATTGCNCC	Macey et al. (2000)	
<b>ND4</b>	ND4	CACCTATGACTACCAAAAGCTCATGTAGA AGC	Modified from Arévalo et al. (1994)*	94 (90), [94 (45), 47 (45), 72 (90) x33], 72 (600)
	leutRNA	AGCCATTACTTTTACTTGGATTTCACC	Modified from Arévalo et al. (1994)*	

\* modified primer sequences developed by Ed Louis, Omaha's Henry Doorly Zoo.

identification using the mitochondrial COI fragment was not possible for some taxa. In these instances, the mitochondrial gene fragments 16S or NADH dehydrogenase subunits 1, 2 and 4 (ND1, ND2, ND4) were amplified and sequenced for a selected number of samples to allow a finer taxonomic identification (see Table S1). For primers and cycling protocols see Table 1. All fragments were sequenced using an ABI 3730XL automated sequencer by Macrogen Inc.

Chromatographs were checked and sequences were edited, where necessary, using the BioEdit sequence alignment editor (version 7.0.5.3; Hall, 1999). To assess the species attribution and the genetic distinctness of each taxa, sequences of each morphological taxa were compared among each other and each sequence was then compared using the BLAST algorithm in GenBank.

Some specimens could not be assigned to any described or identified candidate species as in Vieites et al. (2009), Perl et al. (2014) or Nagy et al. (2012). For these taxa we applied the terms and abbreviations, confirmed candidate species (CCS), unconfirmed candi-

date species (UCS) and deep conspecific lineage (DCL) as defined by Vieites et al. (2009). Working names of the already identified candidate species follow Perl et al. (2014) for amphibians and Nagy et al. (2012) for reptiles. Additionally, when available, we used the names proposed by Glaw and Vences (2007) which usually prefix the species epithet with "sp. aff." of the morphologically closest described species or a descriptor that is either geographic or refers to a characteristic trait of the candidate species. Candidate species of amphibians were identified based on a threshold of 5% minimum divergence for the 16S fragment (Vences et al., 2005a; Fouquet et al., 2007; Vieites et al., 2009), whereas candidate species of reptiles were identified following the different thresholds proposed for the different groups as in Nagy et al. (2012). Obtained sequences were submitted to GenBank (Accession Numbers are available in Table S1) and reptile COI sequences were associated to the BOLD database.

Automated acoustic recording took place at 37 locations. Recordings were made with a single Song Meter SM2 digital recorder (Wildlife Acoustics Inc,

Table 2. Distribution of amphibian and reptile species of the Sahamalaza Peninsula. The survey at Analavory Forest was conducted by Raselimanana (2008), while previous surveys at Anabohazo Forest and Betsimipoaka village were conducted by Andreone et al. (2001). The most recent survey conducted in 2011-13 revisited Anabohazo Forest and Betsimipoaka village, and also surveyed Ankarafa Forest and Antafiabe village and its surroundings. \* species ID limited to photographic record. † species ID limited to observation only. CCS: confirmed candidate species, UCS: unconfirmed candidate species, DCL: deep conspecific lineage (according to Vieites et al., 2009).

	Authority	Analavory		Betsimipoaka		Anabohazo		Ankarafa	Antafiabe
		1996	2000	2013	2000	2011-13	2011-13	2011	
<b>Amphibians</b>									
<i>Aglyptodactylus securifer</i>	Glaw et al., 1998			+	+	+		+	
<i>Blommersia</i> sp. Ca05 (UCS)				+			+	+	
<i>Boophis ankarafensis</i>	Penny et al., 2014								+
<i>Boophis brachychir</i>	(Boettger, 1882)						+	+	+
<i>Boophis jaegeri</i>	Glaw & Vences, 1992					+	+	+	
<i>Boophis tephraeomystax</i>	(Duméril, 1853)	+		+			+	+	+
<i>Boophis tsilomaro</i>	Vences et al., 2010					+	+		
<i>Cophyla berara</i>	Vences et al., 2005					+	+	+	+
<i>Gephyromantis pseudoasper</i>	(Guibé, 1974)					+	+		
<i>Heterixalus luteostriatus</i>	(Andersson, 1910)			+	+		+	+	
<i>Heterixalus tricolor</i> *	(Boettger, 1881)								+
<i>Hoplobatrachus tigerinus</i>	(Daudin, 1803)						+	+	+
<i>Laliostoma labrosum</i> *	(Cope, 1868)	+							
<i>Mantella ebenau</i>	(Boettger, 1880)			+		+	+	+	
<i>Mantidactylus ulcerosus</i>	(Boettger, 1880)			+		+	+	+	+
<i>Platypelis</i> sp. (UCS)*						+			
<i>Ptychadena mascareniensis</i>	(Duméril & Bibron, 1841)	+		+			+	+	+
<i>Rhombophryne</i> sp. (UCS)*						+			
<i>Stumpffia gimmeli</i> *	Glaw & Vences, 1992					+	+	+	
<i>Stumpffia</i> sp. aff. <i>pygmaea</i> Ca "Sahamalaza" (UCS)							+	+	
<b>Reptiles</b>									
<i>Acrantophis madagascariensis</i> *	(Duméril & Bibron, 1844)						+	+	
<i>Alluaudina bellyi</i>	Mocquard, 1894					+			
<i>Amphiglossus reticulatus</i>	(Kaudern, 1922)	+				+	+	+	
<i>Blaesodactylus ambonihazo</i>	Bauer et al., 2011	+							+
<i>Brookesia minima</i>	Boettger, 1893								+
<i>Brookesia stumpffi</i>	Boettger, 1894					+	+	+	
<i>Crocodylus niloticus</i> †	Laurenti, 1768								+
<i>Dromicodryas bernieri</i> *	(Duméril et al., 1854)						+	+	
<i>Dromicodryas quadrilineatus</i> *	(Duméril et al., 1854)	+				+	+	+	+

	Authority	Analavory	Betsimipoaka		Anabohazo		Ankarafa	Antafiabe
		1996	2000	2013	2000	2011-13	2011-13	2011
<i>Ebenavia inunguis</i> (clade Cb)	Boettger, 1878						+	
<i>Flexiseps tanysona</i> *	(Andreone & Greer, 2002)				+		+	
<i>Furcifer oustaleti</i>	(Mocquard, 1894)	+	+			+	+	+
<i>Furcifer pardalis</i>	(Cuvier, 1829)	+			+	+	+	+
<i>Geckolepis humbloti</i>	Vaillant, 1887	+			+	+	+	
<i>Geckolepis</i> sp. aff. <i>maculata</i> (OTU A; CCS)						+		
<i>Hemidactylus mercatorius</i>	Gray, 1842		+	+			+	+
<i>Ithycyphus miniatus</i>	(Schlegel, 1837)				+			
<i>Ithycyphus perineti</i> *	Domergue, 1986					+	+	
<i>Leioheterodon madagascariensis</i> *	Duméril & Bibron, 1854	+	+			+	+	+
<i>Liophidium torquatum</i> *	(Boulenger, 1888)				+	+	+	
<i>Lycodryas granuliceps</i>	(Boettger, 1877)				+	+		
<i>Lygodactylus tolampyae</i>	(Grandidier, 1872)	+			+	+	+	
<i>Madagascarophis colubrinus</i>	(Schlegel, 1837)	+			+	+	+	
<i>Madascincus stumpffi</i>	(Boettger, 1882)				+	+	+	
<i>Mimophis mahfalensis</i> *	(Grandidier, 1867)	+				+	+	+
<i>Oplurus cuvieri</i>	(Gray, 1831)	+	+	+		+	+	+
<i>Paracontias hildebrandti</i>	(Peters, 1880)	+						
<i>Paroedura oviceps</i> *	(Boettger, 1881)				+	+		
<i>Paroedura stumpffi</i>	(Boettger, 1879)				+	+		
<i>Pelomedusa subrufa</i>	(Lacépède, 1788)			+			+	
<i>Phelsuma abbotti</i> *	Stejneger, 1893	+			+			+
<i>Phelsuma kochi</i>	Mertens, 1954	+	+		+	+	+	+
<i>Phelsuma laticauda</i> *	(Boettger, 1880)						+	
<i>Phelsuma</i> sp. aff. <i>quadriocellata</i> (UCS)*							+	
<i>Phelsuma vanheygeni</i> *	Lerner, 2004						+	
<i>Pseudoacantias menamainty</i> *	Andreone & Greer, 2002				+			
<i>Sanzinia madagascariensis</i>	(Duméril & Bibron, 1844)	+			+			
<i>Thamnosophis lateralis</i> *	(Duméril et al., 1854)	+			+	+		+
<i>Trachylepis elegans</i> *	(Peters, 1854)	+	+			+	+	
<i>Trachylepis gravenhorstii</i> (lineage 1, DCL)	(Duméril & Bibron, 1839)				+	+	+	+
<i>Uroplatus ebenau</i>	Boettger, 1879				+	+	+	
<i>Uroplatus henkeli</i>	Böhme & Ibsch, 1990	+			+	+	+	
<i>Zonosaurus laticadatus</i>	(Grandidier, 1869)	+			+	+	+	+

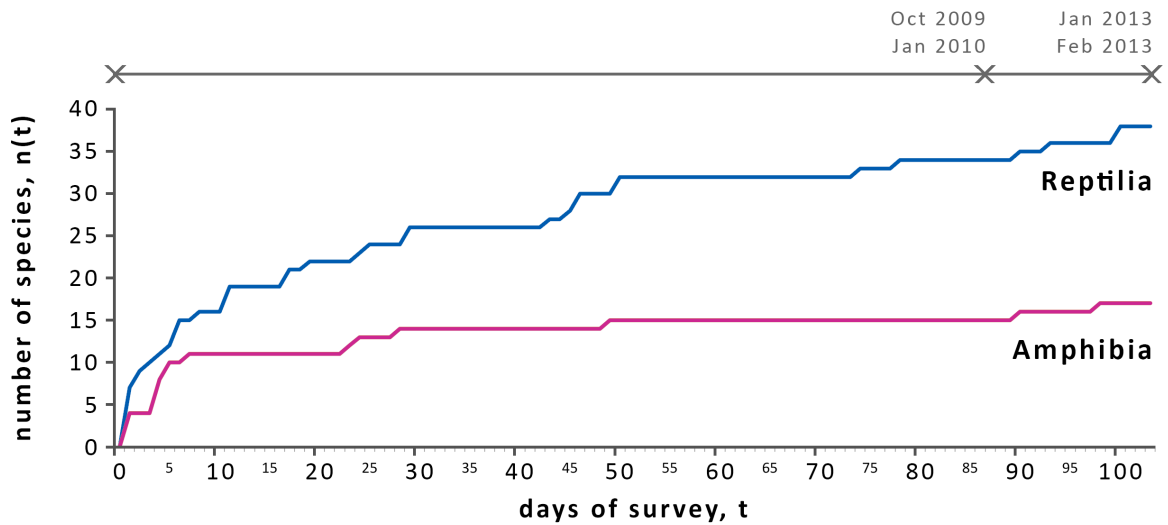


Figure 2. Species accumulation curves (based on all sampling techniques) for amphibian and reptiles species in Sahamalaza Peninsula. Curves show the accumulation during the full duration of the project, covering the wet season.

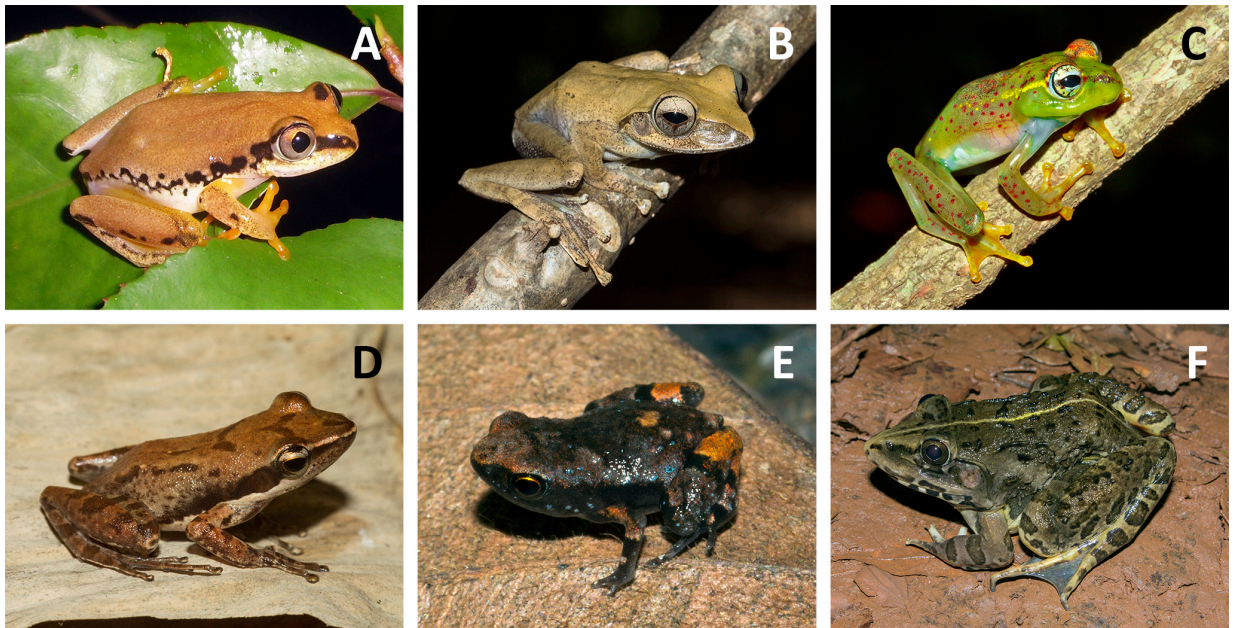


Figure 3. Amphibian species documented for the first time from the Sahamalaza Peninsula during the survey period 2011-2013: **A.** *Heterixalus tricolor* from Ankarafa Forest; **B.** *Boophis brachyichir* from Ankarafa Forest; **C.** *Boophis ankarafensis* recently described from Ankarafa Forest; **D.** *Blommersia* sp. Ca05 (UCS), a candidate species reported from Ankarafa Forest; **E.** *Stumpffia* sp. aff. *pygmaea* Ca “Sahamalaza” (UCS), a candidate species reported from Ankarafa Forest; **F.** *Hoplobatrachus tigerinus* from Anketsakely. Photo A by S. Penny, B-F by G. M. Rosa.





Figure 4. Reptile species documented for the first time from the Sahamalaza Peninsula during the survey period 2011-2013: **A.** *Acrantophis madagascariensis* from Berara Forest; **B.** *Ithycyphus perineti* from Ankarafa Forest; **C.** *Dromicodryas bernieri* from Ankarafa Forest; **D.** *Ebenavia inunguis* from Ankarafa Forest; **E.** *Phelsuma* sp. aff. *quadriocellata* from Ankarafa Forest; **F.** *Phelsuma vanheygeni* from Ankarafa Forest; **G.** *Phelsuma laticauda* from Ankarafa Forest; **H.** *Geckolepis humboldti* from Anketsakely; **I.** *Brookesia minima* from Ankarafa Forest; **J.** *Pelomedusa subrufa* from Betsimipoaka. Photos A-C, E-F by S. Penny, and D, G-J by G. M. Rosa.

Concord, USA) at a 16-bit resolution and 16 kHz sampling rate using two side-mounted SMX-II microphones. The digital recorder was placed one to two metres above the ground/water by securing it with bungee leads to deadwood or a protruding branch. Acoustic recordings were made between sunset and sunrise over 60 nights, when frog activity is greatest (Glaw and Vences, 2007). Continuous recordings split into sections of 120 minutes each were saved in the standard uncompressed .WAV format. Preceding analysis recordings were split using a custom-written MATLAB (The Mathworks, Natick, USA, V7.14.0.739) script into minute long segments to allow for more efficient analysis. Spectrograms were viewed individually as a dual channel output using Avisoft SASLab Pro (Berlin, Germany, V5.2.06); a Hamming window with FFT window size of 512, with 100% frame, and an intensity threshold of 50% were used to create spectrograms. Species were distinguished by matching their temporal and spectral patterns with that of known reference recordings (S. Penny) and an acoustic library of Malagasy frogs (Vences et al., 2006; Rosa et al., 2011). This was achieved by both ear and through taking parameter measurements with Avisoft SASLab Pro (Avisoft SASLab Pro; Berlin, Germany; V5.2.06).

## Results

This survey increases the total number of amphibian and reptile species known from the Sahamalaza Peninsula to 20 and 43 respectively (Table 2). A total of 17 amphibian species and 38 reptile species were found during the current survey period; all were encountered during either opportunistic or transect searching, 14 of the amphibian species were also detected through automated acoustic recording (40122 minutes analysed) and one species of amphibian and three of reptile during pitfall trapping (840 pitfall trap days which yielded a capture rate of 1.12%). Survey effort amounts to 84 days in Ankarafa and 16 days in Anabohazo, or 28 days if survey effort by Andreone et al. (2001) is included. The cumulative number of species detected rose quickly during the start of the survey period and then began to stabilise, with almost 82% of the species being found in the first 50 days of survey (Figure 2). After reaching a plateau, we observed again the discovery of new species during the last 13 days (Figure 2). This overall pattern is observed on both groups of species.

Six species of amphibians (Figure 3) and eleven reptiles (Figure 4) were documented for the first time from

Sahamalaza (Table 2), with two of these taxa qualifying as new candidate species. These are *Boophis ankarafensis*, already described previously as a direct result of this survey (Penny et al., 2014) and *Stumpffia* sp. aff. *pygmaea* Ca “Sahamalaza” (UCS) (Table 2 and S1).

Seven taxa are so far known exclusively from the peninsula: *Boophis ankarafensis*, *Boophis tsilomaro*, *Cophyla berara*, *Platypelis* sp., *Rhombophryne* sp., *Stumpffia* sp. aff. *pygmaea* Ca “Sahamalaza” and *Pseudoacontias menamainty*; although also *Lygodactylus tolampyae* show a distinct genetic distance from the other known locality from where genetic data are available (12% uncorrected pairwise genetic distance at 16S fragment between the population from Sahamalaza and Ankarafantsika) and future taxonomic revisions of this genus might confirm this record as a further candidate new species. There were several species previously recorded from Sahamalaza that were not detected during this last survey: three amphibians (*Laliostoma labrosum*, *Platypelis* sp. and *Rhombophryne* sp.) and five reptiles (*Alluaudina bellyi*, *Ithycyphus miniatus*, *Paracontias hildebrandti*, *Pseudoacontias menamainty* and *Sanzinia madagascariensis*) (see Table 2).

Nine of the species in our survey are treated as synonymous with those identified by Andreone et al. (2001), these are: *Boophis tsilomaro* (with *Boophis albilabris*), *Cophyla berara* (with *Cophyla* sp. 12), *Stumpffia gimmeli* (with *Stumpffia* cf. *gimmeli*), *Flexiseps tanysona* (with *Amphiglossus* sp.), *Phelsuma kochi* (with *Phelsuma madagascariensis*), *Pseudoacontias menamainty* (with *Pseudoacontias* n. sp.), *Blaesodactylus ambonihazo* (with *Blaesodactylus sakalava*), *Hemidactylus mercatorius* (with *H.* cf. *frenatus*) and *Lycodryas granuliceps* (with *L. pseudogranuliceps*).

## Discussion

### Survey effort

Sampling techniques varied in efficiency. All species were detected during either opportunistic or transect searching and we consider this to be the most efficient survey methods. Pitfall trapping contributed the fewest number of specimens and proved ineffective at capturing amphibians, which are often proficient climbers or strong jumpers, enabling them to escape; the technique was more useful for the detection of fossorial reptile species, although all species we detected through pitfall trapping were also identified through other sam-

pling techniques. However, in 2000 they enabled the discovery of the so far only known specimen of *Pseudioacantias menamainty*. Automated acoustic recording allowed for the rapid detection of amphibian species within a habitat; however as amphibian vocalisations are usually limited to the breeding period (Glaw and Vences, 2007) seasonally in-active species will have been missed. Existing audio reference recordings were required to correctly pair a vocalisation to a species during the analysis stage, thus automated acoustic recording must be used in tandem with other sampling methods to avoid missing the vocalisations of undescribed or unknown taxa. Thus, in our opinion this technique is more suited to habitat surveys for areas where most species are already known, rather than species inventories in limited surveyed areas. Despite providing no unique species records compared to the other techniques, unlike pitfall trapping, it required minimal field effort and enabled the expansion of monitoring to areas that may otherwise have been missed. We thus consider it to be a very useful tool for herpetological surveys.

#### *Species composition of the Sahamalaza Peninsula*

Although surveys always depend on contingency, it is likely that a significant proportion of Sahamalaza's amphibian and reptile fauna have been detected, when considering all herpetological survey work of the area. The detection of three new taxa (*Boophis ankarafensis*, *Stumpffia* sp. aff. *pygmaea* Ca "Sahamalaza", *Geckolepis humboldti*) unnoticed during the previous surveys, highlights the efficiency of using an integrative approach to species identification. The detection of several species (almost 20%) in the final few weeks of the wet season, together with the detection of species missed during a previous survey by Andreone et al. (2001), highlights the necessity of conducting herpetological surveys over extended periods for areas with strong seasonal differences.

The presence of species representative of the drier biomes of West Madagascar (e.g. *Aglyptodactylus securifer*, *Blommersia* sp. Ca05 (UCS), *Heterixalus luteostriatus*, *H. tricolor*, *Laliostoma labrosum*, *Blaesodactylus ambonihazo*, *Oplurus cuvieri*, *Madascincus stumpffi* and *Zonosaurus laticaudatus* concurrent with species representative of the rainforests of Sambirano region to the north (e.g. *Boophis brachyichir*, *B. jaegeri*, *B. tephraeomystax*, *Gephyromantis pseudoasasper*, *Mantella ebenau*, *Mantidactylus ulcerosus*, *Stumpffia gimmeli*, *Alluaudina bellyi*, *Brookesia*

*stumpffi*, *B. minima*, *Ebenavia inunguis*, *Ithycyphus perinetti*, *Phelsuma laticauda*, *P. vanheygeni*, *Paroedura oviceps*, *P. stumpffi*, *Uroplatus henkeli* and *U. ebenau*) confirms that Sahamalaza's intermediate climate supports a transitional fauna between these two biomes.

The two forests of Anabohazo and Ankarafa show broadly similar species compositions with a few notable differences (Table 2). Four amphibian and ten reptile species were recorded in Anabohazo Forest but not in Ankarafa, while two amphibian and eight reptile species were found in Ankarafa but not in Anabohazo. It is likely that some of these differences only reflect bias in survey effort between the two locations. For example, several of the Gekkonidae detected from Ankarafa and not in Anabohazo: *Blaesodactylus ambonihazo*, *E. inunguis*, *Phelsuma* sp. aff. *quadriocellata* and *P. vanheygeni*, were likely missed due to the shorter time spent surveying this area, coupled with their infrequent to rare encounter rates. On the other hand, the species recorded in Anabohazo but not in Ankarafa are prone to have been missed, due to the positive bias in the sampling period in Ankarafa. However, the two forests fragments differ in size, habitat quality and geography and so some differences in species composition might be due to this. The two fragments are separated from one another by around 20 km of savannah and scrubland, potentially isolating many of the forest-dependent species. The far-ranging calls of *G. pseudoasper* were extremely conspicuous throughout Anabohazo yet entirely absent from Ankarafa. This difference cannot be attributed to season as surveys in Ankarafa took place immediately before and after the sampling period in Anabohazo. Anabohazo marks the most southerly extent of this species range (Glaw and Vences, 2007) and it is possible that the climate or geography of Ankarafa make it unsuitable for *G. pseudoasper*. The recently described *Boophis ankarafensis* was only found along perennial lotic streams in Ankarafa, a hydrological feature that is entirely absent within Anabohazo, which may explain its potential absence from here. This factor likely accounts for the non record of the helmeted turtle *Pelomedusa subrufa*.

#### *Range extensions*

All species documented from Ankarafa Forest represent new records from this locality. Several species were recorded from Sahamalaza for the first time and represent important range extensions. For *Boophis tsi-*

*lomaro*, *Cophyla berara* and *Blaesodactylus ambonihazo* we provide the first distribution record outside of their respective type localities. In the case of *Boophis tsilomaro* and *Cophyla berara* the range expansion is still limited to the Sahamalaza Peninsula. Yet, it is worth noting that in *C. berara* we observe a genetic distance of 1% between the two known populations of Berara and Ankarafa. The record of *Blaesodactylus ambonihazo* represents a significant increase in its distributional range (extended northward by over 200 km; Bauer et al., 2011; Ikeuchi and Mori, 2014). The recent formal description of this species (Bauer et al., 2011), along with those of *B. victori* Ineich et al., 2016 and *B. microtuberculatus* Jono et al., 2015, together with the confirmed sympatry of *B. victori* with *B. sakalava* (Grandidier, 1867), and of *B. microtuberculatus* with *B. boivini* Duméril, 1856 (Jono et al., 2015; Ineich et al., 2016), further highlights the importance of applying a taxonomically integrative approach, and the need to reassess previously known localities and providing new genetic data. The distribution of *B. ambonihazo* may extend to other dry forests fragments in north-western Madagascar, but due to the genera's apparent requirements for areas of relatively low disturbance containing at least some large trees, its distribution is likely to be severely fragmented (Ineich et al., 2016).

The species *Acrantophis madagascariensis* and *Crocodylus niloticus* are reported from Sahamalaza for the first time. Unfortunately, *Acrantophis madagascariensis* was only recorded in the 2011-2012 expedition and no genetic data are available on this record. Only a single specimen of *C. niloticus* was sighted, of which the tail-end was seen slipping into the water of the Vavan'aneno River in Antafiabe; local people attested the presence of multiple specimens within the area but note that the largest individuals have been lost to hunting. The presence of the snake *Ithycyphus perineti*, gecko *Ebenavia inunguis*, chameleon *Brookesia minima* and treefrog *Boophis brachyichir* within Sahamalaza extend their ranges over 100 km south along Madagascar's west coast from Nosy Be (Glaw and Vences, 2007). The population of the *Ebenavia inunguis* sampled in Ankarafa belong to the Clade Cb (*sensu* Hawlitschek et al., 2017) as the population from Nosy Be, that is the type locality of this taxon. These two populations have a genetic distance of 4% at the analysed COI fragment, and thus far this represent the only other record for this taxon outside of Nosy Be. *Brookesia minima* was known at least from Nosy Be and Manongarivo and the population sampled in this study has a genetic distance of 5% with the population

from Manongarivo. Similarly, *B. brachyichir* was already reported for Nosy Be, Manongarivo, Forêt d'Ambre and near Antsiranana. This record thus represent the southern most new distribution.

The presence of *Heterixalus tricolor* confirms the species' distribution between Nosy Be and Ankarafantsika (Glaw and Vences, 2007). The presence of the turtle *Pelomedusa subrufa* extends their range over 200 km northeast of a record from Mahajanga (Iverson, 1992; Glaw and Vences, 2007; Petzold et al., 2014), placing this population at the northern edge of their projected distribution (Boycott and Bourquin, 2008), although no genetic distance was observed between the *P. subrufa* sequences of the newly reported population and the available sequences in Genbank.

We treated *Phelsuma kochi* as synonymous with *P. madagascariensis*, recorded by Andreone et al. (2001) following molecular identification; however, a photo from the earlier survey period resembles *P. grandis* Gray, 1870, known from the Sambirano region to the north, and it remains possible that the species occurs in sympatry with *P. kochi*. The occurrence of *P. kochi* extends their range over 200 km northeast of Ankarafantsika (Mori et al., 2006; Glaw et al., 2011) and the genetic distance between the population from Sahamalaza and Tsingy de Bemaraha is of 7% at the analysed COI fragment.

This survey documents the first record of *Phelsuma laticauda* from Sahamalaza, a species known from a number of locations across northern Madagascar (Gelach et al., 2011). The presence of *Phelsuma vanheygeni* increases their known range of about 50 km south beyond the Ampasindava peninsula, where the species was classified as Endangered due to their restricted range (Randrianantoandro et al., 2011). The presence of *Phelsuma* sp. aff. *quadriocellata* marks their only documented occurrence in north western Madagascar and a significant distance from the populations known from Eastern Madagascar (Glaw and Vences, 2007). Furthermore, individual's from Sahamalaza occurred at heights of 150-170 m asl, significantly lower than the mid-elevation areas of 720-1350 m asl where the species is generally reported in the East (Glaw and Vences, 2011). The individuals encountered were found residing in *Pandanus* screw palms, a trait shared with *Phelsuma quadriocellata* (Peters 1883), however it is unknown whether they are truly conspecific as genetic data are not available. Their rare encounter rate from Sahamalaza may mean they have been missed by other surveys and indicate the species occurs between these distant sites; alternatively, they

may belong to a different *P.* species. The species may be synonymous with *Phelsuma* cf. *quadriocellata* reported from Nosy Be (Andreone et al., 2003). The record of *Madascincus stumpffi* in Sahamalaza, similar with the record from Marojejy, mark the southernmost distributional record for the species, but the population from Sahamalaza have a genetic distance of 9% at the analysed COI fragment if compared with the population of *M. stumpffi* of Forest d'Ambre.

The presence of the frog *Blommersia* sp. Ca05 (UCS) marks a range increase of over 300 km beyond Tsingy de Bemaraha. Populations are also known from Isalo, Makay and Kirindy, while recent records of a *Blommersia* species from Mariarano and Mitsinjo near the Besiboka delta may also be attributed to *B.* sp. Ca05 (Rakotoarison et al., 2015), potentially indicating the species is widely distributed along the Madagascar's west coast. Finally, we report a new record of the recently resurrected *Geckolepis humboldti* which, in Madagascar, was until now known only in the Tsingy de Bemaraha. The newly reported population of *Geckolepis humboldti* from Sahamalaza has a genetic distance of 8-9% with the populations from the Comoros and Mayotte.

#### Endemicity patterns

Two new species of amphibians (*B. ankarafensis* and *Stumpffia* sp. aff. *pygmaea* Ca "Sahamalaza" (UCS)) identified in this survey and four species identified in prior surveys (*Boophis tsilomaro*, *Cophyla berara*, *Rhombophryne* sp. and *Platypelis* sp.) may represent local endemics as they have not been detected in other surveys of Northwest Madagascar (e.g. Nosy Be, Manongarivo, Tsaratanana, Benavony), in some cases, despite their prominent and distinctive calls (Vences et al., 2005b, 2010b; Glaw and Vences, 2007). The failure to detect neither *Platypelis* sp. nor *Rhombophryne* sp. during the most recent surveys mean that further effort should be invested in the area, as representatives of these genera are sometimes very difficult to detect. The population of *Lygodactylus tolampyae* from Sahamalaza was already known, however this population has a high genetic divergence with the other population of this species for which genetic data are available. A more in depth taxonomic revision of this genus is needed to apply this name to a specific taxon, until then it will not be possible to assess the taxonomic identification of the *Lygodactylus tolampyae* population from Sahamalaza. However, this might represent a new microendemic species of reptile along with the pre-

viously identified and highly elusive skink *Pseudoacantias menamainty*. All this points towards the Sahamalaza peninsula being an important centre of microendemicity.

The new species of treefrog, *Boophis ankarafensis*, was described following the results of this survey (Penny et al., 2014). The species is only known from the banks of perennial streams in intact forest vegetation in Ankarafa Forest and has been classified as Critically Endangered on the IUCN Red List. The new candidate species, *Stumpffia* sp. aff. *pygmaea* Ca "Sahamalaza" (UCS), still awaits formal description but molecular data found only a 92-93% match (*p*-distance transformed into percent; at the analysed 16S fragment) with *S. pygmaea* and their taxonomic distinctness seems therefore to be granted. The species produces inconspicuous calls from within leaf-litter which are difficult to locate, thus the species may have been missed during surveys outside the peninsula. On the contrary, this is such a small amphibian species that dispersal capacities might be very low. Our survey expands the range of *Boophis tsilomaro* beyond their type locality of Berara. The detection of *B. tsilomaro* from Anketsakely, a fragment of forest within Anabohazo, contributes only a marginal increase in range, and the species is confined to an area of less than 5 km<sup>2</sup>, qualifying it as Critically Endangered. The species' absence from Ankarafa Forest, the only other significant area of forest on the peninsula, reinforces the importance of protecting all remaining areas of natural habitat in Sahamalaza, as populations may be reliant on particular conditions.

This survey expands the range of *Cophyla berara* beyond their type locality of Berara: a fragment of primary forest in Anabohazo (Vences et al., 2005b). We document the species throughout the fragments of Ankarafa Forest, the surroundings of Antafiabe village and the fragment of Anketsakely in Anabohazo Forest. These locations are no greater than 20 km distant from the type locality, yet mark an important extension to the distribution of this species and indicate multiple populations exist. Furthermore, *C. berara* were found in abundance in low quality secondary forest, a habitat common throughout the peninsula. Secondary tracts of regenerating forest are one of the most common forest types in Ankarafa and past land clearances have created a matrix of interlinked forest fragments surrounded by large thickets of bamboo. *C. berara* were extremely abundant in these forest edge habitats, and in interior sections where bamboo were present, a habit also reported in *C. maharipeo* (Rakotoarison et al.,

2015). This association is likely due to their breeding habitat of laying spawn inside water-filled segments of bamboo. The species was detected in all surveyed forest fragments, including isolated sections of heavily degraded forest that had experienced recent burning; callers were also found perched on scorched leaves and branches. Thus, this species seems to be adapted to disturbed forest, and is less likely to experience severe decline in the immediate future. However, its long-term viability in these small isolated forest fragments is unknown and even with these new range extensions, it is still known from just three areas within the Sahamalaza Peninsula, which itself totals around 26000 hectares. There appears to be limited gene flow between populations in Ankarafa and Anabobahazo and molecular analyses show they have already slightly diverged, with two fix substitutions at the analysed mitochondrial 16S fragment (Penny et al., 2016). Although the species appears relatively well adapted to disturbed forest, it is still a forest-dependent species and at risk from future habitat destruction.

#### Threats and conservation

Forest on the peninsula continues to be exploited by the human populations. Fire has already destroyed Analavory Forest, leaving Ankarafa and Anabobahazo the largest areas of intact forest in Sahamalaza. These two locations are subject to high levels of forest clearance to make way for crop cultivation and pastureland (Penny et al., 2014, 2016). Furthermore, fires lit in the dry season to rejuvenate grazing land frequently spread out of control and burn adjacent areas of intact forest. Selective logging of tropical hardwoods and small-scale quarrying were also observed in Anabobahazo Forest, although currently this is still the more intact of the two remaining forests. If actions, such as those outlined by a recently published conservation action plan on the amphibians of Sahamalaza (Penny et al., 2016) are not promptly implemented, then all the peninsula's forest dwelling herpetofauna will suffer serious population declines and the local endemics will be pushed towards extinction.

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## Supplementary Information

Table S1. List of all samples for which DNA sequences were produced in this study. For each sample we indicate sample ID, species name, collection locality and GenBank accession number \$, New range extension.

Sample ID	Species	Locality	16S	COI	ND1	ND2	ND4
<b>Amphibians</b>							
ACP1215	<i>Aglyptodactylus securifer</i>	Sahamalaza Peninsula	Anketsakely	MG189395			
ACP1226	<i>Aglyptodactylus securifer</i>	Sahamalaza Peninsula	Anketsakely	MG189396			
ACP1230	<i>Aglyptodactylus securifer</i>	Sahamalaza Peninsula	Anketsakely	MG189397			
ACP1244	<i>Aglyptodactylus securifer</i>	Sahamalaza Peninsula	Betsimipoaka	MG189398			
ACP1063	<i>Blommersia</i> sp. Ca05 (UCS)	Sahamalaza Peninsula	Ankarafa \$	MG189399			
ACP1064	<i>Blommersia</i> sp. Ca05 (UCS)	Sahamalaza Peninsula	Ankarafa \$	MG189400			
ACP1140	<i>Blommersia</i> sp. Ca05 (UCS)	Sahamalaza Peninsula	Ankarafa \$	MG189401			
ACP1175	<i>Blommersia</i> sp. Ca05 (UCS)	Sahamalaza Peninsula	Ankarafa \$	MG189402			
ACP1179	<i>Blommersia</i> sp. Ca05 (UCS)	Sahamalaza Peninsula	Ankarafa \$	MG189403			
ACP1197	<i>Blommersia</i> sp. Ca05 (UCS)	Sahamalaza Peninsula	Anketsakely \$	MG189404			
ACP1146	<i>Blommersia</i> sp. Ca05 (UCS)	Sahamalaza Peninsula	Ankarafa \$	MG189405			
ACP1152	<i>Blommersia</i> sp. Ca05 (UCS)	Sahamalaza Peninsula	Ankarafa \$	MG189406			
ACP1153	<i>Blommersia</i> sp. Ca05 (UCS)	Sahamalaza Peninsula	Ankarafa \$	MG189407			
ACP1165	<i>Blommersia</i> sp. Ca05 (UCS)	Sahamalaza Peninsula	Ankarafa \$	MG189408			
ACP1061	<i>Boophis ankarafensis</i>	Sahamalaza Peninsula	Ankarafa	MG189409			
ACP1062	<i>Boophis ankarafensis</i>	Sahamalaza Peninsula	Ankarafa	MG189410			
ACP1185	<i>Boophis ankarafensis</i>	Sahamalaza Peninsula	Ankarafa	MG189411			
ACP1186	<i>Boophis ankarafensis</i>	Sahamalaza Peninsula	Ankarafa	MG189412			
ACP1184	<i>Boophis jaegeri</i>	Sahamalaza Peninsula	Ankarafa	MG189413			
ACP1193	<i>Boophis jaegeri</i>	Sahamalaza Peninsula	Anketsakely	MG189414			
ACP1194	<i>Boophis jaegeri</i>	Sahamalaza Peninsula	Anketsakely	MG189415			

Sample ID	Species	Locality	16S	COI	ND1	ND2	ND4
ACP1196	<i>Boophis jaegeri</i>	Sahamalaza Peninsula	Anketsakely	MG189416			
ACP1218	<i>Boophis jaegeri</i>	Sahamalaza Peninsula	Anketsakely	MG189417			
ACP1148	<i>Boophis brachychir</i>	Sahamalaza Peninsula	Ankarafa \$	MG189418			
ACP1149	<i>Boophis brachychir</i>	Sahamalaza Peninsula	Ankarafa \$	MG189419			
ACP1150	<i>Boophis brachychir</i>	Sahamalaza Peninsula	Ankarafa \$	MG189420			
ACP1177	<i>Boophis brachychir</i>	Sahamalaza Peninsula	Ankarafa \$	MG189421			
ACP1192	<i>Boophis brachychir</i>	Sahamalaza Peninsula	Anketsakely \$	MG189422			
ACP1195	<i>Boophis brachychir</i>	Sahamalaza Peninsula	Anketsakely \$	MG189423			
ACP1221	<i>Boophis brachychir</i>	Sahamalaza Peninsula	Anketsakely \$	MG189424			
ACP1163	<i>Boophis tephraeomystax</i>	Sahamalaza Peninsula	Ankarafa	MG189425			
ACP1158	<i>Boophis tephraeomystax</i>	Sahamalaza Peninsula	Ankarafa	MG189426			
ACP1167	<i>Boophis tephraeomystax</i>	Sahamalaza Peninsula	Ankarafa	MG189427			
ACP1245	<i>Boophis tephraeomystax</i>	Sahamalaza Peninsula	Betsimipoaka	MG189428			
ACP1235	<i>Boophis tephraeomystax</i>	Sahamalaza Peninsula	Anketsakely	MG189429			
ACP1219	<i>Boophis tsilomaro</i>	Sahamalaza Peninsula	Anketsakely \$	MG189430			
ACP1224	<i>Boophis tsilomaro</i>	Sahamalaza Peninsula	Anketsakely \$	MG189431			
ACP1227	<i>Boophis tsilomaro</i>	Sahamalaza Peninsula	Anketsakely \$	MG189432			
ACP1237	<i>Boophis tsilomaro</i>	Sahamalaza Peninsula	Anketsakely \$	MG189433			
ACP1138	<i>Cophyla berara</i>	Sahamalaza Peninsula	Ankarafa \$	MG189434			
ACP1657	<i>Cophyla berara</i>	Sahamalaza Peninsula	Sahamalaza \$	MG189435			
ACP1157	<i>Cophyla berara</i>	Sahamalaza Peninsula	Ankarafa \$	MG189436			
ACP1168	<i>Cophyla berara</i>	Sahamalaza Peninsula	Ankarafa \$	MG189437			
ACP1204	<i>Cophyla berara</i>	Sahamalaza Peninsula	Berara	MG189438			
ACP1205	<i>Cophyla berara</i>	Sahamalaza Peninsula	Berara	MG189439			
ACP1212	<i>Cophyla berara</i>	Sahamalaza Peninsula	Berara	MG189440			
ACP1214	<i>Cophyla berara</i>	Sahamalaza Peninsula	Berara	MG189441			

Sample ID	Species	Locality		16S	COI	ND1	ND2	ND4
ACP1172	<i>Cophyla berara</i>	Sahamalaza Peninsula	Ankarafa \$	MG189442				
ACP1173	<i>Cophyla berara</i>	Sahamalaza Peninsula	Ankarafa \$	MG189443				
ACP1174	<i>Cophyla berara</i>	Sahamalaza Peninsula	Ankarafa \$	MG189444				
ACP1217	<i>Gephyromantis pseudoasper</i>	Sahamalaza Peninsula	Berara	MG189445				
ACP1187	<i>Heterixalus luteostriatus</i>	Sahamalaza Peninsula	Ankarafa	MG189446				
ACP1220	<i>Heterixalus luteostriatus</i>	Sahamalaza Peninsula	Anketsakely	MG189447				
ACP1228	<i>Heterixalus luteostriatus</i>	Sahamalaza Peninsula	Anketsakely	MG189448				
ACP1242	<i>Heterixalus luteostriatus</i>	Sahamalaza Peninsula	Betsimipoaka	MG189449				
ACP1232	<i>Hoplobatrachus tigerinus</i>	Sahamalaza Peninsula	Anketsakely	MG189450				
ACP1246	<i>Mantella ebenau</i>	Sahamalaza Peninsula	Anketsakely	MG189451				
ACP1178	<i>Mantella ebenau</i>	Sahamalaza Peninsula	Ankarafa	MG189452				
ACP1203	<i>Mantella ebenau</i>	Sahamalaza Peninsula	Berara	MG189453				
ACP1139	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189454				
ACP1141	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189455				
ACP1144	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189456				
ACP1145	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189457				
ACP1154	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189458				
ACP1155	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189459				
ACP1164	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189460				
ACP1166	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189461				
ACP1171	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189462				
ACP1176	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189463				
ACP1189	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Ankarafa	MG189464				
ACP1191	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Anketsakely	MG189465				
ACP1200	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Anketsakely	MG189466				
ACP1201	<i>Mantidactylus ulcerosus</i>	Sahamalaza Peninsula	Anketsakely	MG189467				

Sample ID	Species	Locality	16S	COI	ND1	ND2	ND4
ACP1247	<i>Ptychadena mascareniensis</i>	Sahamalaza Peninsula	Betsimipoaka	MG189468	MG189476		
ACP1160	<i>Stumpffia</i> sp. aff. <i>pygmaea</i> Ca “Sahamalaza” (UCS)	Sahamalaza Peninsula	Ankarafa	MG189469			
ACP1161	<i>Stumpffia</i> sp. aff. <i>pygmaea</i> Ca “Sahamalaza” (UCS)	Sahamalaza Peninsula	Ankarafa	MG189470			
ACP1162	<i>Stumpffia</i> sp. aff. <i>pygmaea</i> Ca “Sahamalaza” (UCS)	Sahamalaza Peninsula	Ankarafa	MG189471			
ACP1199	<i>Stumpffia</i> sp. aff. <i>pygmaea</i> Ca “Sahamalaza” (UCS)	Sahamalaza Peninsula	Anketsakely	MG189472			
<b>Reptiles</b>							
ACP1188	<i>Amphiglossus reticulatus</i>	Sahamalaza Peninsula	Ankarafa		MG189477		
ACP1238	<i>Amphiglossus reticulatus</i>	Sahamalaza Peninsula	Anketsakely		MG189478		
ACP1169	<i>Blaesodactylus ambonihazo</i>	Sahamalaza Peninsula	Ankarafa \$		MG189479		
ACP1159	<i>Brookesia minima</i>	Sahamalaza Peninsula	Ankarafa \$		MG189480	MG189539	
ACP1181	<i>Brookesia minima</i>	Sahamalaza Peninsula	Ankarafa \$		MG189481	MG189540	
ACP2751	<i>Brookesia minima</i>	Sahamalaza Peninsula	Ankarafa \$		MG189482		
ACP1202	<i>Brookesia stumpffi</i>	Sahamalaza Peninsula	Berara		MG189483		
ACP1222	<i>Brookesia stumpffi</i>	Sahamalaza Peninsula	Anketsakely		MG189484		
ACP1207	<i>Brookesia stumpffi</i>	Sahamalaza Peninsula	Anketsakely		MG189485		
ACP1209	<i>Brookesia stumpffi</i>	Sahamalaza Peninsula	Berara		MG189486		
ACP1156	<i>Ebenavia inunguis</i> (clade Cb)	Sahamalaza Peninsula	Ankarafa \$		MG189487		
ACP1183	<i>Ebenavia inunguis</i> (clade Cb)	Sahamalaza Peninsula	Ankarafa \$		MG189488		
ACP1170	<i>Furcifer oustaleti</i>	Sahamalaza Peninsula	Ankarafa		MG189489		
ACP1190	<i>Furcifer oustaleti</i>	Sahamalaza Peninsula	Anketsakely		MG189490		
ACP1210	<i>Furcifer pardalis</i>	Sahamalaza Peninsula	Berara		MG189491		
ACP1236	<i>Furcifer pardalis</i>	Sahamalaza Peninsula	Anketsakely		MG189492		
ACP1198	<i>Furcifer pardalis</i>	Sahamalaza Peninsula	Anketsakely		MG189493		
ACP1216	<i>Geckolepis humboldti</i>	Sahamalaza Peninsula	Anketsakely \$		MG189494		MG189526
ACP1680	<i>Geckolepis humboldti</i>	Sahamalaza Peninsula	Sahamalaza \$		MG189495		MG189527

Sample ID	Species	Locality	16S	COI	ND1	ND2	ND4
ACP2531	<i>Geckolepis</i> sp. aff. <i>maculata</i> (OTU A; CCS)	Sahamalaza Peninsula	Anketsakely		MG189496		MG189528
ACP1143	<i>Hemidactylus mercatorius</i>	Sahamalaza Peninsula	Ankarafa		MG189497		
ACP1142	<i>Lygodactylus tolampyae</i>	Sahamalaza Peninsula	Ankarafa		MG189498		MG189529
ACP1180	<i>Lygodactylus tolampyae</i>	Sahamalaza Peninsula	Ankarafa		MG189499		MG189530
ACP2749	<i>Lygodactylus tolampyae</i>	Sahamalaza Peninsula	Ankarafa	MG189473	MG189500		
ACP2750	<i>Lygodactylus tolampyae</i>	Sahamalaza Peninsula	Ankarafa	MG189474	MG189501		
ACP1208	<i>Madagascarophis colubrinus</i>	Sahamalaza Peninsula	Anketsakely		MG189502		
ACP1213	<i>Madagascarophis colubrinus</i>	Sahamalaza Peninsula	Berara		MG189503		
ACP1240	<i>Madagascarophis colubrinus</i>	Sahamalaza Peninsula	Anketsakely		MG189504		
ACP1241	<i>Madagascarophis colubrinus</i>	Sahamalaza Peninsula	Anketsakely	MG189475	MG189505		
ACP1234	<i>Madascincus stumpffi</i>	Sahamalaza Peninsula	Anketsakely \$		MG189506	MG189536	
ACP1681	<i>Madascincus stumpffi</i>	Sahamalaza Peninsula	Sahamalaza \$		MG189507	MG189537	
ACP1147	<i>Oplurus cuvieri</i>	Sahamalaza Peninsula	Ankarafa		MG189508		MG189531
ACP1239	<i>Paroedura stumpffi</i>	Sahamalaza Peninsula	Anketsakely		MG189509		
ACP1682	<i>Paroedura stumpffi</i>	Sahamalaza Peninsula	Sahamalaza		MG189510		
ACP1243	<i>Pelomedusa subrufa</i>	Sahamalaza Peninsula	Betsimipoaka \$		MG189511		
ACP1182	<i>Phelsuma kochi</i>	Sahamalaza Peninsula	Ankarafa \$		MG189512		
ACP1233	<i>Lycodryas granuliceps</i>	Sahamalaza Peninsula	Anketsakely		MG189513		
ACP1223	<i>Trachylepis gravenhorstii</i> (lineage 1, DCL)	Sahamalaza Peninsula	Anketsakely		MG189514		
ACP1151	<i>Uroplatus ebenau</i>	Sahamalaza Peninsula	Ankarafa				MG189532
ACP1211	<i>Uroplatus ebenau</i>	Sahamalaza Peninsula	Berara				MG189533
ACP1206	<i>Uroplatus henkeli</i>	Sahamalaza Peninsula	Anketsakely		MG189515		MG189525
ACP1225	<i>Uroplatus henkeli</i>	Sahamalaza Peninsula	Anketsakely		MG189516		MG189534
ACP1231	<i>Uroplatus henkeli</i>	Sahamalaza Peninsula	Anketsakely		MG189517		MG189535
ACP1229	<i>Zonossaurus laticaudatus</i>	Sahamalaza Peninsula	Anketsakely		MG189518	MG189538	

