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From Ground to Canopy: A Molecularly Verified Herpetofaunal Inventory of a Madagascar Protected Area

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ABSTRACT.—Madagascar faces extensive deforestation and habitat loss that threaten its endemic herpetofauna. However, many Malagasy reptile and amphibian species lack basic natural history data (e.g., vertical habitat use) and reliable information on their geographic distribution (i.e., data verified by mitochondrial barcodes), which could inform conservation efforts. These efforts are especially important in isolated patches of remnant forests, such as the Ivohiboro Protected Area, which may act as a refuge for imperiled endemic species. Using both vertical and horizontal sampling methods in Ivohiboro, Madagascar, combined with a molecular characterization of encountered specimens via sequences of the mitochondrial 16S rRNA gene, we recorded a total of 32 reptile and 22 anuran species, including one newly described species of *Boophis*, one candidate species of *Anodonthyla*, and a recently described *Guibemantis* species. By surveying from ground to canopy, we found anuran abundance and richness to be strongly weighted toward the ground, possibly because of dry conditions during sampling periods, whereas reptiles were found consistently across the full vertical gradient. qPCR-based screening of skin swabs from 362 frog specimens did not reveal evidence for the presence of the fungal pathogen *Batrachochytrium dendrobatidis* at Ivohiboro. Our biodiversity inventory contributes to ongoing discoveries at Ivohiboro and underscores an urgent need for conservation measures to protect this unique ecosystem. Additionally, the discrepancy we observed between DNA-based identification of species and field identification demonstrates the importance of DNA sampling approaches to obtain reliable faunal inventories in poorly studied regions that often harbor numerous morphologically cryptic species.

Madagascar is a globally significant biodiversity hotspot (Myers et al., 2000; Myers, 2003), hosting numerous endemic taxa because of its approximately 87 million years of evolutionary isolation (Storey et al., 1995). Indeed, the island's natural geography and geology provide the basis for a wide variety of ecosystems and climatic environments, ranging from mountainous tropical rainforests to expansive drylands (Antonelli et al., 2022). Madagascar's native flora and fauna are under severe pressure from human-induced land use and climate change (Antonelli et al., 2022). Long-term deforestation has resulted in extensive loss of tropical forest area on Madagascar's east coast, imperiling numerous endemic species (Harper et al., 2007). At the same time, Madagascar ranks 177th out of 193 countries on the Human Development Index (United Nations Development Programme, 2023), resulting in a scarcity of funds for research on the biology, ecology, and systematics of its unique biota. To curb further biodiversity loss and protect native flora and fauna of Madagascar, it is critical to inventory and catalog ecosystems across the island as a foundational step to inform effective conservation actions, guide priority setting, and support decision-making.

Amphibians and reptiles in particular represent one of the most threatened yet understudied components of Madagascar's biodiversity (Glaw et al., 2022). The island is home to hundreds of endemic frog, chameleon, and gecko species, among others, yet most lack basic natural history data, including vertical niche use. Vertical niche data beyond categorical delineations (e.g., arboreal, terrestrial) are globally deficient for herpetofauna,

with only a small number of studies having investigated fine-scale vertical distribution of amphibians (e.g., Philippines: Scheffers et al., 2013; Madagascar: Basham et al., 2019; Panama: Basham and Scheffers, 2020; Gabon: Basham et al., 2024) and reptiles (e.g., Philippines: Gonzalez et al., 2020). Indeed, in many tropical forests, the fraction of amphibian and reptile species that are arboreal is regularly above 50% (Kays and Allison, 2001); thus, this important range metric is missing for most species. In Madagascar, many species of frogs are arboreal but have only been observed during their breeding activity in and near water bodies (Glaw and Vences, 2007), and their broader ecology, including vertical niche use, remains almost completely unknown.

Threats to amphibians include habitat loss, climate change, and pathogens, particularly *Batrachochytrium dendrobatidis* (*Bd*), which causes the disease chytridiomycosis in susceptible amphibians (Fisher and Garner, 2020). This chytrid fungus, particularly the Global Panzootic Lineage (*Bd*-GPL), has spread to many of the world's amphibian biodiversity hotspots, causing mortality events in many regions because of rapid onset and high transmissibility of chytridiomycosis (Lips, 2016). Whereas some studies have characterized chytridiomycosis as a globally catastrophic threat to amphibians (e.g., Scheele et al., 2019), others have questioned the extent and generalizability of its impact across regions and taxa (Lambert et al., 2020). In Madagascar, this complexity is further reflected in an ongoing debate: Multiple laboratories have reported *Bd*-GPL-like positive samples from wild amphibians, yet no chytridiomycosis symptoms or mass mortality events have been observed (Bletz et al., 2015, 2022; Kolby and Skerratt, 2015). Therefore, efforts must be directed toward ascertaining and monitoring the presence of *Bd*

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in Madagascar and its effects on Malagasy amphibians, which are known to be susceptible to chytridiomycosis through lab studies (Bletz et al., 2015; Kolby et al., 2015).

A striking characteristic of Madagascar's biota, including the herpetofauna, is a high proportion of microendemic species (Wilmé et al., 2006; Brown et al., 2016), defined as range-restricted species that often also have small areas of occupancy. (Steigerwald et al., 2024). Because forests have largely disappeared in many parts of Madagascar, these microendemic species are now in some cases restricted to very small remaining isolates of their original habitat, surrounded by a matrix of unsuitable land. These remnant isolated forests, even if occupying only small areas, are therefore of high conservation value in Madagascar. The Ivohiboro Protected Area (IPA) is one such forest managed by a consortium composed of the Malagasy Institut pour la Conservation des Écosystèmes Tropicaux (MICET), the biological station Centre ValBio, the Rainforest Trust, and stakeholders from surrounding villages (Otero Jimenez et al., 2023). Although it has been highly fragmented by years of anthropogenic disturbance, the ~850 ha of humid forest within IPA retains high levels of biodiversity, with numerous species of amphibians, reptiles, birds, lemurs, invertebrates, and other taxa (Bartylak et al., 2022; Fahmy et al., 2023; Otero Jimenez et al., 2023). Given its small size and high conservation value, the IPA's humid forest serves as a microreserve at a time when the number of new amphibian-containing microreserves has been in decline over the last two decades (Steigerwald et al., 2024). In the same general area of Madagascar, several other isolated forest fragments have yielded remarkable herpetofaunal discoveries, including microendemic species (Belluardo et al., 2021).

Here, we report an intensive herpetological inventory of the IPA forest, including systematic vertical and horizontal sampling that encompassed a range of forest habitats. Identification of encountered amphibians and reptiles was refined using DNA barcodes. Furthermore, to aid national monitoring of *Bd*, we also surveyed for its presence. Our study provides evidence for a rich and *Bd*-free amphibian and reptile community at IPA, including several newly discovered candidate species and regional microendemics, which highlights the importance of this isolated forest for conservation.

MATERIALS AND METHODS

Study Sites.—We surveyed the IPA, a 3,782 ha area in south-eastern Madagascar that encompasses approximately 858 ha of humid forest located on an isolated range of quartzite hills (centered on geographical coordinates 22.5996 °S, 46.7187 °E; WGS84; 650–800 m a.s.l., Figs. 1A, B). The area around Ivohiboro is characterized by a matrix of savanna grasslands, xeric forests, and isolated hilltop forest fragments (Figs. 1C–E), with a wet-warm season from November to March and a dry-cool season from April to October.

Herpetofauna Sampling.—For our inventory, we combined herpetofauna records from multiple sources that used different methods. We conducted vertical visual encounter surveys in 2016 and 2023, and we also made incidental sightings and ground-based surveys between 2016 and 2023. A previous study used incidental data collected prior to 2020 to provide a preliminary inventory, which we refined and enriched here (Otero Jimenez et al., 2023). We surveyed across two subsites within the IPA, known as Ivohibory (north) and Analamary (south) (Fig. 1). We conducted mitochondrial barcoding on amphibians sampled in 2023.

We used vertical surveys in two separate sampling periods: December 2016 and October–November 2023. Although sampling periods were scheduled at the beginning of the wet-warm season, the sampling took place under unusually dry conditions because the dry season continued longer than expected in both years. Each vertical survey centered on a single canopy tree, following previously published methods (Basham and Scheffers, 2020). At night we surveyed 16 trees in 2016 and 26 trees in 2023, with no repeat surveys of the same tree. At each tree, one surveyor searched from ground to canopy for reptiles and amphibians, with a 4-m diameter circle surveyed at ground level to standardize the search area available during climbing. In addition, a ground surveyor searched a 10-m radius circle around the tree over 60 min to supplement data for the overall inventory. We measured the height above ground and distance to the survey centroid of each captured individual using a laser distance meter (Leica Geosystems, Leica Disto D2; <http://www.leica-geosystems.ca>).

Laboratory Methods.—We photographed, measured, and weighed all encountered amphibians and reptiles. In addition, we swabbed all captured frogs after removal of small debris via a brief rinse with purified water produced from a Grayl UltraPress®. We conducted frog swabbing with a sterile, rayon-tipped swab (MW113, Medical Wire & Equipment), which was streaked back and forth in an anterior-posterior direction 5 times on each ventral thigh and foot as well as 10 times on the venter and dorsum. We stored swabs in 1.5 ml Eppendorf tubes with 1 mL of 96% EtOH. We stored samples in a cool shady area for several days until refrigeration at 4 °C was possible, at which point we kept samples at approximately 4 °C until processing. To prevent cross-contamination, we kept each frog in a separate bag for field processing and we wore a fresh pair of powder-free nitrile gloves or inverted bags to manipulate each frog (Weldon et al., 2013). We identified reptiles by morphology using morphological features as summarized in the field guide (Glaw and Vences, 2007) and subsequent taxonomic revisions, whereas we first identified amphibians by morphology and then refined identifications by DNA barcoding. Because of complex permitting constraints associated with the 2023 fieldwork—conducted as part of a large, multinational, multi-taxa expedition—we were unfortunately unable to collect specimens, which would have significantly enhanced the impact of this research (Vonesh et al., 2009). However, all genetic data collected are available via the repository detailed below.

We extracted DNA from swabs using the Qiagen DNeasy Blood and Tissue Pro Kit according to the Animal Tissues protocol (Qiagen, Germantown, Maryland, USA). We tested DNA extracts from 362 individuals for presence and prevalence of *Bd*. For qPCR, we employed a TaqMan assay (Boyle et al., 2004), with 1.2 µM each of primers ITS1-3 Chytr and 5.8S Chytr. These primers target a 146-bp region of the ITS-1 region of the *Bd* ribosomal operon. We used Chytr MGB2 as our minor groove binder probe. We added BSA to prevent PCR inhibition. We ran qPCRs on a QuantStudio™ 3 (Applied Biosystems). Cycling conditions were as follows: one 20 s Taq activation step at 95 °C, 50 cycles of 95 °C for 1 s, and 60 °C for 20 s. For all qPCRs, we used a serial dilution series of synthetic gBlocks standards of the ITS region from 10⁷–10¹ ITS copies. We ran two replicates for each sample and repeated samples that were positive in one of the two replicates with a further two replicates. We considered a replicate positive if it amplified before 50 cycles with an ITS copy number greater than 0. We considered at least three positives out of four replicates to indicate a potential positive result for *Bd*. We included three negative

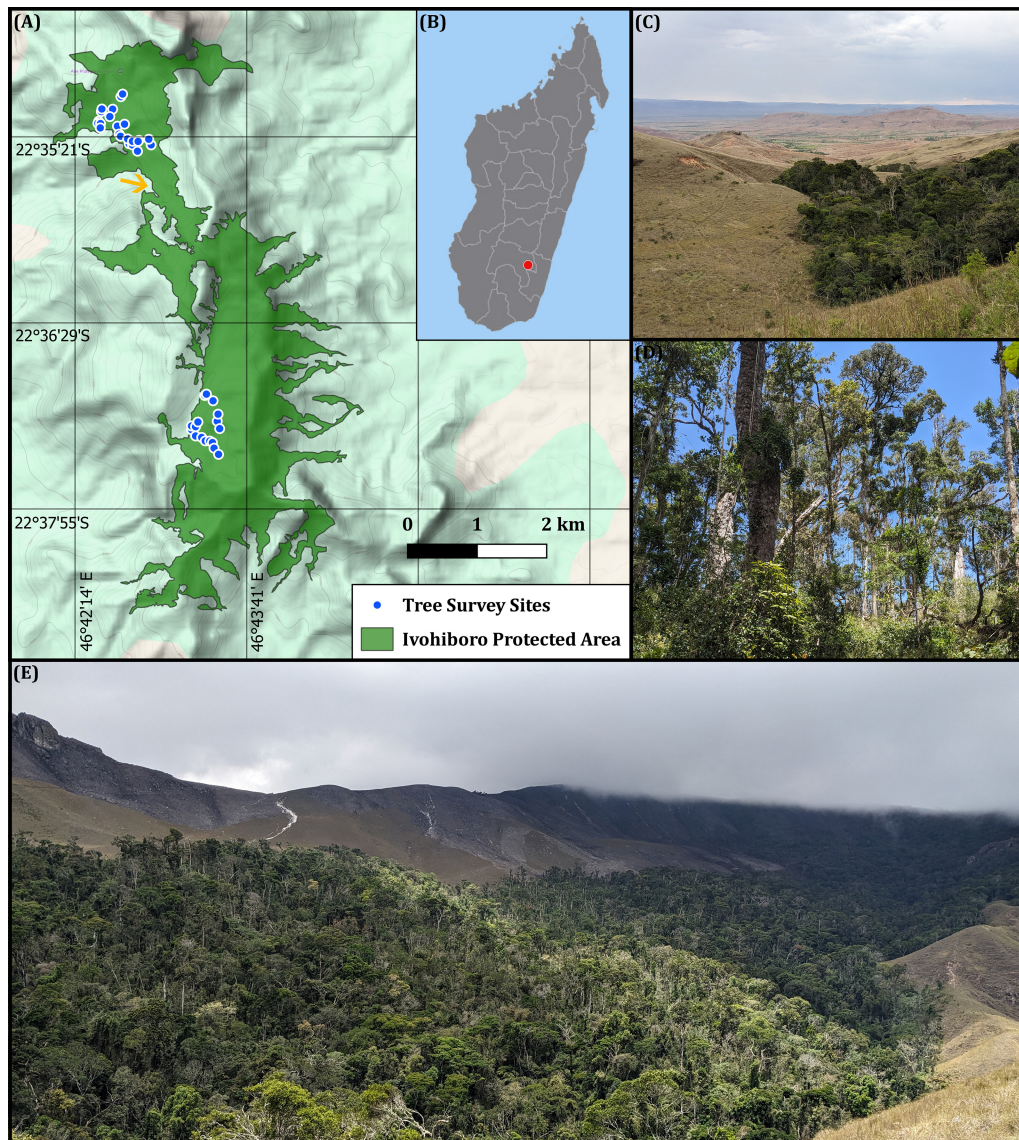


FIG. 1. Map of tree survey sites at IPA (A), with an inset of Madagascar showing the site location (B). The orange arrow in (A) represents the direction of the photographer when taking photograph (E). (C–E) Photos of the habitat at Ivohiboro.

controls to check for possible contamination during all qPCRs in which no template DNA was added. We retested samples considered positive using dPCR, where each reaction comprised a 10 μ L volume, consisting of 2 μ L of AbsoluteQ dPCR Master Mix (5X) (Applied Biosystems, Inc.), 0.5 μ L of Chytr MGB2 Assay (see Boyle et al., 2004) at a 20X concentration (equal volumes of 18 μ M of ITS-1 Chytr3 primer, 18 μ M 5.8S Chytr primer, and 5 μ M of Chytr MGB2 probe), 6.4 μ L of nuclease-free water, and 1.1 μ L of template DNA. We pre-loaded 9 μ L of the reaction mix into different wells on a MAP16 Plate (A52865, Applied Biosystems, Inc.) and then overlaid each reaction with 15 μ L of QuantStudio AbsoluteQ Isolation Buffer (Applied Biosystems, Inc.). We set the cycling parameters as follows: initial denaturation at 96 $^{\circ}$ C for 10 min, followed by 50 cycles of 96 $^{\circ}$ C for 5 s and 60 $^{\circ}$ C for 15 s. We ran positive controls (22 and 220 zoospore genomic equivalents; strain JEL423) and negative control reactions. We conducted all genotyping reactions using the QuantStudio Absolute Q Digital PCR System (Applied Biosystems, Inc.) and performed subsequent data analysis using the QuantStudio AbsoluteQ Digital PCR Software on a Windows PC.

To identify individual frogs to species, we processed DNA extracts of swabs from 201 individuals through PCR amplification of a ~550-bp segment of the 16S ribosomal gene. We used the 16SA-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16SBH-NEW (5'-CCTGGATTACTCCGGTCTGA-3) forward and reverse primers, respectively (Palumbi et al., 1991). We performed amplifications in a reaction volume of 12.5 μ L with the following conditions: 94 $^{\circ}$ C for 90 s, followed by 33 cycles: 94 $^{\circ}$ C for 45 s, 55 $^{\circ}$ C for 45 s, and 72 $^{\circ}$ C for 90 s. We sent PCR products for purification and Sanger sequencing at Eton Bio (San Diego, California 92121). We manually edited sequences recovered for quality in CodonCode Aligner (CodonCode Corp.). For molecular identification of samples to species, we followed a two-step protocol. First, we compared sequences against a curated reference library of all available sequenced amphibian species of Madagascar. For this purpose, we aligned IPA sequences and reference sequences using the FFT-NS-1 approach in MAFFT (Kato and Standley, 2013) as implemented in Concatenator (Vences et al., 2022), reconstructed a maximum likelihood tree, and calculated uncorrected pairwise distances in MEGA v7

(Kumar et al., 2016). We considered sequences unambiguously clustering with reference sequences and separated by short branch lengths (<3% uncorrected pairwise distances) to be reliably identified to species. We compared other sequences to the full set of reference sequences available in GenBank via BLAST searches. All newly determined sequences from IPA were submitted to GenBank (accession numbers PV386798–PV386957). A spreadsheet containing metadata of recorded specimens, links to relevant photos, and DNA sequences is available from the Zenodo repository (<https://doi.org/10.5281/zenodo.17235865>).

We used the DNA barcode-based identifications to refine and modify identification of records from nonbarcoded specimens such as those reported by Otero Jimenez et al. (2023) and from any other available photographs from previous expeditions (Table 1). For example, where we found morphological field identification to consistently differ from the more reliable molecular identification for the sequenced specimens, we renamed the sequenced as well as other individuals in the prior data sets accordingly. We, therefore, consider our records to be of three broad confidence levels: (1) fully reliable—16S DNA barcoded and the molecular identification cross-checked with morphology, (2) highly reliable—not DNA barcoded but reliably identified from photographs, and (3) moderately reliable—a sighting (i.e., listed in Otero Jimenez et al. 2023), confirmed by MV as plausible, but with no photos or habitat information available.

Statistical Analysis.—We conducted all analyses using R Statistical Software (R Core Team, 2025). Using only data collected from vertical surveys, we used the “geom_density” function (kernel = gaussian; bandwidth = 2) within the “ggplot2” package (Wickham, 2016) to visualize relative density of capture heights for reptiles and amphibians. To test whether reptiles and amphibians differed significantly in their mean vertical distribution, we conducted a one-way analysis of variance (ANOVA) comparing mean capture height between the two taxonomic groups. We assessed community richness and composition across vertical height using a site (1-m height intervals) by species matrix, which included species found during both vertical and ground surveys. The first height category was for individuals captured at 0 m (i.e., site 1), then >0 and ≤1 m (site 2), >1 and ≤2 m (site 3), and so on to 30 m (site 31). We removed singletons (to reduce the impact of rare species whose vertical range was not fully sampled), transformed the data to presence/absence (to isolate composition without abundance), and filled gaps between the lowest and highest records for each species (representing the range where the species would be expected to occur). We also tested whether vertical height influenced detection (i.e., distance of detected individuals from the survey centroid) using a linear regression (distance~height).

RESULTS

Richness and Abundance.—Combined with data from Otero Jimenez et al. (2023), the total number of amphibian species at the three levels of confidence cumulatively was 20, 21, and 22 species, and for reptiles, 0, 25, and 32 species (Table 1; Figs. 2, 3, 4). For amphibians, both richness and abundance were greater nearer to the ground (Figs. 5A, C), with very few amphibians found above the understory stratum. In contrast, reptiles were found throughout the vertical profile of the forest, particularly *Uroplatus* geckos and *Calumma* chameleons (Figs. 5B, C). Reptiles occurred significantly higher

in the canopy (mean = 7.84 m ± 0.61 SE, $n = 84$) than amphibians (mean = 0.64 m ± 0.21 SE, $n = 45$; ANOVA: $F_{1,127} = 70.95$, $P < 0.001$), with most amphibians (51%) found at ground level (0 m) compared to a median height of 6.5 m for reptiles. There was no effect of vertical height on horizontal detection distance (slope = 0.0029, $P = 0.91$, $R^2 < 0.001$), suggesting that detection probability did not vary systematically with height under our standardized search protocol. However, we cannot rule out the possibility that variation in structural complexity across heights may have influenced detection probability.

Sequencing revealed limitations in identification when relying on the pictorial field guide (Glaw and Vences, 2007), because of intraspecific variability, lack of available morphological descriptions and natural history data for many Malagasy herpetofauna, and numerous taxonomic changes that were published post-2007. For example, *Boophis goudoti* and *B. periegetes* appeared to both be present in the IPA based on field identification alone, but when sequenced, all specimens were revealed to be *B. obscurus*, a species with a variable color pattern that is known to be common in the general geographical area. Similarly, *Mantidactylus biporus*, *M. mocquardi*, *M. curtus*, and *M. alutus* were revealed to be a single polymorphic species, *M. bourgati*.

Bd.—A single sample tested partially positive for *Bd*, with three of four qPCR replicates returning weak positive amplification. However, subsequent analysis using the more sensitive dPCR assay returned a negative result. While we cannot exclude the possibility that extremely low-level infections may have fallen below the detection threshold of dPCR, the combined evidence, together with the historically low prevalence of *Bd* in the region (Kolby et al., 2015), suggests that this signal most likely represents a false positive.

New Candidate Species Discoveries and Range Expansions.—Mitochondrial DNA barcoding identified three lineages of taxonomic interest within the genera *Anodonthis* (Fig. 3), *Boophis* (Fig. 4), and *Guibemantis* (Fig. 2). Two of these correspond to confirmed or recently described species. The *Boophis* lineage is now described as *Boophis samuelsabini* from the type locality of Ivohiboro (Basham et al., 2026). In our data set, this species was most closely related to *B. roseipalmatus* (4.7% uncorrected pairwise mtDNA divergence) and *B. madagascariensis* (5.5% divergence), consistent with its placement within the *B. madagascariensis* species group. We recorded 21 specimens of *B. samuelsabini*, including 13 individuals identified by DNA barcoding and 8 identified based on diagnostic morphological characters. The *Anodonthis* lineage corresponds to a confirmed candidate species following the terminology of Vieites et al. (2009), here referred to as *Anodonthis* sp. Ca7. This lineage was most closely related to *A. rouxae* (9.0% uncorrected pairwise divergence). We recorded approximately 13 specimens, including 3 individuals identified by DNA barcoding and an additional 9–10 individuals assigned based on concordant morphology. The *Guibemantis* lineage corresponds to *Guibemantis sioka*, a species recently described by Hutter et al. (2025) and previously known from a single individual collected in Andohahela National Park. Our records, therefore, substantially extend the known distribution and sample size of this species.

Several other herpetofaunal records from IPA are of potential taxonomic and biogeographic interest. The finding of the snake *Sanzinia voluntary* (if confirmed by genetic data in the future) might be one of the easternmost records of this predominantly western species. The available photos indicate that the undertermined *Liophidium* may correspond to one of the rarer species occurring in Madagascar's southwest, such as *Liophidium*

TABLE 1. Summary of amphibian and reptile species recorded from the IPA. Numbers under confidence levels represent the number of individual records assigned to each species. Totals represent the cumulative number of records across all confidence level categories. Confidence level 1=individuals identified using 16S DNA barcoding and cross-checked with morphology (fully reliable); confidence level 2=individuals identified from photographs or morphology without DNA barcoding (highly reliable); confidence level 3=plausible sighting records from previous surveys lacking photographs or genetic confirmation (moderately reliable). *=species for which previous surveys reported presence only, without abundance information.

Taxonomy				Records by confidence level			
Main clade	Family	Genus	Species	1	2	3	Totals
Squamata	Chamaeleonidae	<i>Calumma</i>	<i>andringitraense</i>		3	9	12
Squamata	Chamaeleonidae	<i>Calumma</i>	<i>crypticum</i>		3	23	26
Squamata	Chamaeleonidae	<i>Calumma</i>	<i>nasutum/fallax complex</i>		6	58	64
Squamata	Chamaeleonidae	<i>Calumma</i>	<i>oshaughmessyi</i>		1	10	11
Squamata	Chamaeleonidae	<i>Furcifer</i>	<i>lateralis</i>			1	1
Squamata	Chamaeleonidae	<i>Furcifer</i>	<i>oustaleti</i>			*	
Squamata	Chamaeleonidae	<i>Palleon</i>	<i>nasus</i>			4	4
Squamata	Gekkonidae	<i>Hemidactylus</i>	<i>mercatorius</i>			1	1
Squamata	Gekkonidae	<i>Lygodactylus</i>	sp. undetermined		1	19	20
Squamata	Gekkonidae	<i>Paroedura</i>	<i>bastardi complex</i>			4	4
Squamata	Gekkonidae	<i>Phelsuma</i>	<i>lineata</i>			*	
Squamata	Gekkonidae	<i>Phelsuma</i>	<i>quadriocellata</i>		6	2	8
Squamata	Gekkonidae	<i>Uroplatus</i>	<i>malahelo</i>		2	23	25
Squamata	Gekkonidae	<i>Uroplatus</i>	<i>phantasticus</i> or <i>malama</i>			2	2
Squamata	Gekkonidae	<i>Uroplatus</i>	<i>sikorae</i>		2	1	3
Squamata	Opluridae	<i>Oplurus</i>	<i>quadriraculatus</i>			1	1
Squamata	Gerrhosauridae	<i>Zonosaurus</i>	sp. undetermined			*	
Squamata	Scincidae	<i>Flexisepe</i>	cf. <i>ornaticeps</i>			1	1
Squamata	Scincidae	<i>Madascincus</i>	<i>ankodabensis</i>		1	1	2
Squamata	Scincidae	<i>Madascincus</i>	<i>pyrurus</i>			1	
Squamata	Scincidae	<i>Trachylepis</i>	<i>elegans</i>			1	1
Squamata	Scincidae	<i>Trachylepis</i>	<i>gravenhorstii</i>			1	1
Squamata	Scincidae	<i>Trachylepis</i>	<i>vato</i>			1	1
Squamata	Boidae	<i>Acrantophis</i>	<i>dumerili</i>			*	
Squamata	Lamprophiidae	<i>Compsophis</i>	<i>infralineatus</i>		3	4	7
Squamata	Lamprophiidae	<i>Madagascarchophis</i>	<i>meridionalis</i> or <i>colubrinus</i>			*	
Squamata	Lamprophiidae	<i>Liophidium</i>	sp. undetermined			1	1
Squamata	Typhlopidae	<i>Madatyphlops</i>	<i>arenarius</i>			1	1
Squamata	Lamprophiidae	<i>Mimophis</i>	<i>mahfalensis</i>			*	
Squamata	Boidae	<i>Sanzinia</i>	<i>volontany</i>			1	1
Squamata	Pseudoxyrhophiidae	<i>Dromicodryas</i>	<i>bernieri</i>			1	
Squamata	Pseudoxyrhophiidae	<i>Thamnosophis</i>	<i>lateralis</i>			1	1
Amphibia	Mantellidae	<i>Aglyptodactylus</i>	<i>madagascariensis</i>			6	6
Amphibia	Microhylidae	<i>Anodonthyla</i>	sp. nov. (Ca7)	3	10	2	15
Amphibia	Mantellidae	<i>Boophis</i>	<i>albilabris</i>			*	
Amphibia	Mantellidae	<i>Boophis</i>	<i>andohahela</i>		2	2	4
Amphibia	Mantellidae	<i>Boophis</i>	<i>boppa</i>	6	2	4	12
Amphibia	Mantellidae	<i>Boophis</i>	<i>doulioti</i>			3	3
Amphibia	Mantellidae	<i>Boophis</i>	<i>obscurus</i>	16	37	7	60
Amphibia	Mantellidae	<i>Boophis</i>	<i>samuelsabini</i>	13	8	9	30
Amphibia	Mantellidae	<i>Gephyromantis</i>	<i>blanci</i>			4	4
Amphibia	Mantellidae	<i>Gephyromantis</i>	<i>cornucopia</i>			32	32
Amphibia	Mantellidae	<i>Gephyromantis</i>	<i>spinifer</i>		5	12	17
Amphibia	Mantellidae	<i>Guibemantis</i>	<i>sioka</i>			1	1
Amphibia	Mantellidae	<i>Laliostoma</i>	<i>labrosum</i>			1	1
Amphibia	Mantellidae	<i>Mantella</i>	<i>betsileo</i>			2	2
Amphibia	Mantellidae	<i>Mantidactylus</i>	<i>betsileanus</i>			5	5
Amphibia	Mantellidae	<i>Mantidactylus</i>	<i>bourgati</i>	34	66	3	103
Amphibia	Mantellidae	<i>Mantidactylus</i>	<i>delorme</i>			3	3
Amphibia	Mantellidae	<i>Mantidactylus</i>	<i>femoralis</i>	15	44	28	87
Amphibia	Mantellidae	<i>Mantidactylus</i>	<i>katae</i>			5	5
Amphibia	Mantellidae	<i>Mantidactylus</i>	sp. Ca48	7	8	1	16
Amphibia	Microhylidae	<i>Plethodontohyla</i>	<i>inguinalis</i>			2	2
Amphibia	Ptychadenidae	<i>Ptychadena</i>	<i>mascareniensis</i>		1	1	2

apperti, *L. trilineatum*, or *L. vaillanti*, which can only be verified once a voucher specimen or a genetic sample becomes available. The record of the frog *Boophis boppa* is a southward extension of the range of this poorly known species, which previously had only been recorded from Antoetra and Ranomafana (Hutter

et al., 2018). Co-occurrence of the two frogs *Gephyromantis blanci* and *G. cornucopia* has previously been reported from Pic d'Ivohibe (Miralles et al., 2023) but observing the same pattern at Ivohiboro further confirms the species status of these morphologically similar species.



FIG. 2. A subset of amphibians and reptiles observed at Ivohiboro: (I), *Anodonthyla* sp. nov., (Ca7), (II), *Boophis samuelsabini*, (III), *Boophis andohahela*, (IV), *Boophis boppa*, (V), *Boophis doulioti*, (VI), *Boophis obscurus*, (VII), *Gephyromantis blanci*, (VIII), *Gephyromantis cornucopia*, (IX), *Gephyromantis spinifer*, (X), *Guibemantis sioka* (Ca19), (XI), *Laliostoma labrosum*, (XII), *Mantidactylus* sp. Ca48. Photos © E. B.

DISCUSSION

The discoveries of *Boophis samuelsabini* and a candidate species of *Anodonthyla*, together with the second-ever record of the enigmatic *Guibemantis sioka* exemplify Madagascar's exceptional biodiversity that remains to be explored. Indeed, as of 2009 it was estimated using a comprehensive barcode sampling effort that ~209 undescribed frog species were present in Madagascar, for a total of ~465 species which is ~5% of all anuran species globally (Vieites et al., 2009). Convergent evolution of color patterns and morphological variability of many species have made identification difficult. Indeed, without

genetic methods, individuals of some of the most common species (e.g., *M. bourgati* and *B. obscurus*) found in this study would have been misidentified if relying on the current pictorial guide and local community experts. However, it should be noted that there are limitations to the short 16S rRNA mitochondrial gene barcoding we employed in this study. Despite being widely used for amphibian systematics and species delimitation, short fragments (~500 bp) of 16S have been shown to result in inaccurate phylogenetic reconstructions, with lower and more variable branch support compared to those produced by full-length sequences (Chan et al., 2022). Furthermore, the use of only a single mitochondrial genetic marker cannot detect phenomena



FIG. 3. A subset of amphibians and reptiles observed at Ivohiboro: (I), *Mantidactylus bourgati*, (II), *Mantidactylus delormei*, (III), *Mantidactylus femoralis*, (IV), *Mantidactylus katae*, (V), *Plethodontohyla inguinalis*, (VI), *Calumma andringitraense*, (VII), *Calumma crypticum*, (VIII), *Calumma fallax/nasutum*, (IX), *Calumma oshaughnessyi*, (X), *Compsophis infralineatus*, (XI), *Flexiseps cf. ornateiceps*, (XII), *Furcifer lateralis*. Photos © E. B. apart from (IX) by Alex Wiebe.

such as hybridization and mitochondrial introgression, which may blur the taxonomic signal. Thus, although we found consistently strong support for species delimitations, the full description of the new species—those already described and those subjected to ongoing study—additionally rely on morphology, bioacoustics, and nuclear-encoded genetic markers.

Biogeographically, the herpetofaunal assemblage of Ivohiboro combines species distributed mainly in arid Madagascar, such as the snakes *Madatyphlops arenarius*, *Sanzinia voluntary*, and *Acrantophis dumerili* and the frogs *Laliostoma labrosum* and *Mantella betsileo*, with species typically restricted to highland forests of the

Southern Central East, such as the frogs *M. bourgati*, *M. delormei*, *G. blanci*, and *G. cornucopia*, as well as other more common species from Madagascar's eastern rainforests such as the chameleon *C. crypticum*, the snake *Compsophis infralineatus*, and the frogs *Aglyptodactylus madagascariensis*, *Mantidactylus betsileanus*, *M. katae*, *M. femoralis*, and *Plethodontohyla inguinalis* (see Glaw and Vences, 2007 and subsequent taxonomic revisions for distribution ranges of these species). These patterns highlight the importance of herpetofauna inventories in remaining fragments of Madagascar's highlands (e.g., Belluaro et al., 2021) to better understand the ecotones between Madagascar's main biomes (Brown et al., 2016).



FIG. 4. A subset of amphibians and reptiles observed at Ivohiboro: (I), *Madascincus ankodabensis*, (II), *Madatyphlops arenarius*, (III), *Oplurus quadrimaculatus*, (IV), *Palleon nasus*, (V), *Paroedura bastardi* complex, (VI), *Phelsuma quadriocellata*, (VII), *Thamnosophis lateralis*, (VIII), *Trachylepis elegans*, (IX), *Trachylepis gravenhorstii*, (X), *Trachylepis vato*, (XI), *Uroplatus phantasticus/malama*, (XII), *Uroplatus sikorae*. Photos © E. B. apart from (V, XI, and XII) by Alex Wiebe.

Species exhibited strong differences in vertical niche use across the forest profile. Amphibians were generally restricted to the lower strata, with the greatest abundance and richness at ground level, which declined rapidly in the first 5 m of vertical height (Figs. 5A, C). In contrast, we found a rich reptile community from understory to canopy, primarily made up of chameleons and geckos, as well as skinks at the forest floor and snakes, which differed in vertical niche use by species (Figs. 5B, C). It is likely that some species found only in the understory also occur in the canopy (e.g., *Uroplatus sikorae*, and *U. phantasticus/*

malama); indeed, *Uroplatus* species were also found consistently in the canopy in Ranomafana (E.B., unpubl. data). Importantly, all surveys were conducted during the extended dry season in the region. We hypothesized that frogs, during the wet season, would occur higher in the canopy because they would be released from lower strata which act as a moist refugia during the dry season. Indeed, during three wetter days in 2023 (6–8 November), individuals of the undescribed *Anodonthyla* were found at 10 m height, the highest records for amphibians across all surveys. At Ranomafana National Park, *Anodonthyla* species

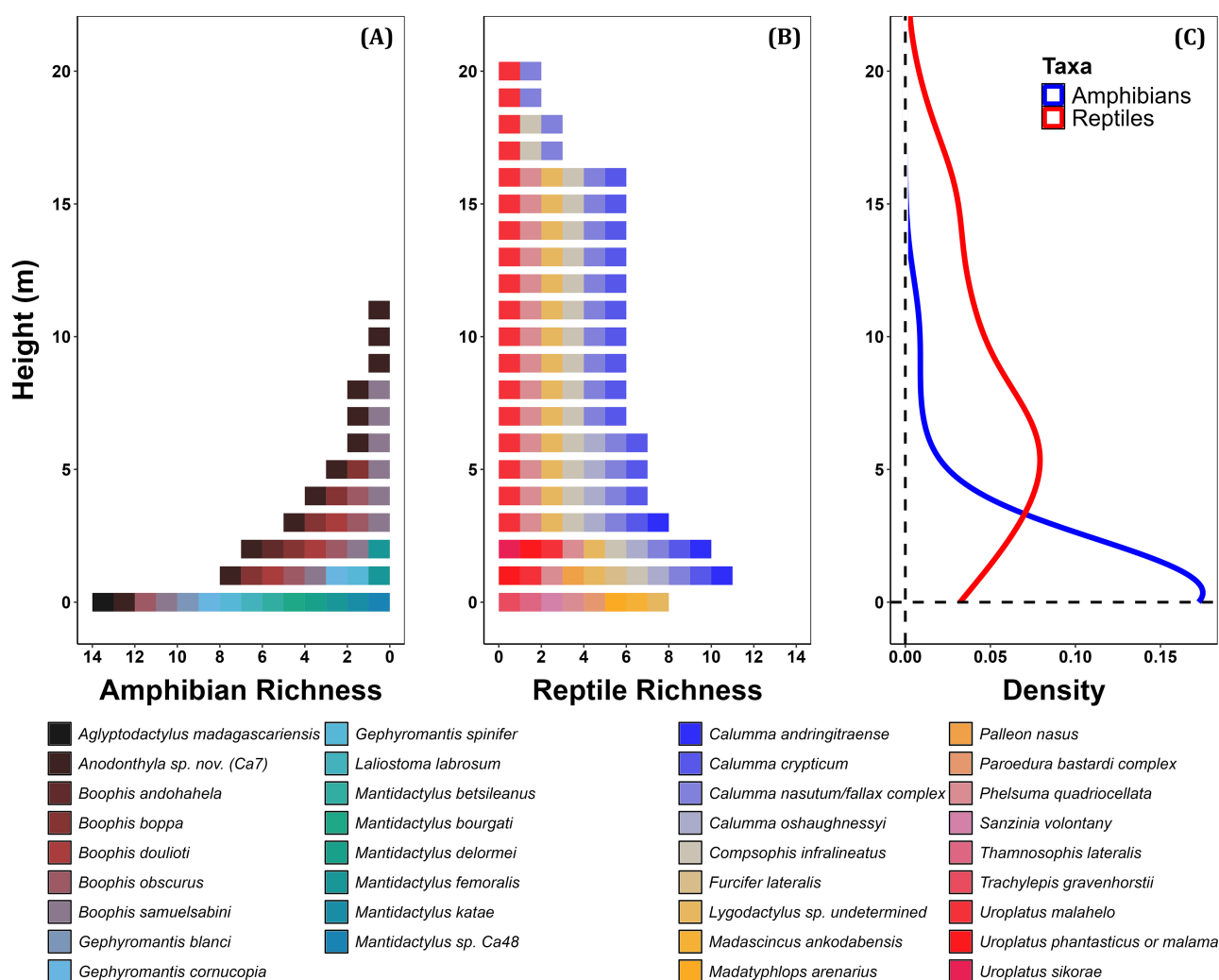


FIG. 5. Vertical stratification of amphibian (A) and reptile (B) richness and composition at Ivohiboro. Vertical density (i.e., relative abundance) of reptiles and amphibians (C).

were regularly found in the high canopy during the wet season, usually inhabiting *Asplenium* fern microhabitats (Basham et al., 2019). Observed patterns of amphibian stratification also closely mirror those seen across seasons in Panama (Basham and Scheffers, 2020), where many species descended to the ground during the dry season. Importantly, climate change is predicted to increase the length and intensity of dry seasons across the next century (Chadwick et al., 2016; Feng et al., 2013; IPCC, 2014; Neelin et al., 2006), likely resulting in a downward shift in community organization across the vertical gradient and a reduction in the reproductive period for many amphibians.

Climate change has been linked to an increase and spread of droughts in southern Madagascar (Rigden et al., 2024). Combined with forest fragmentation and widespread pasture burning for cattle, these droughts have intensified risk of wild-fire and forest degradation (Frappier-Brinton and Lehman, 2022; Desbureaux and Damania, 2018). The IPA itself sits atop an elevated range of hills and serves as a critical watershed for downstream communities. Despite this ecological importance, the greatest threat to the site remains fire, fueled by traditional fire-use practices in the lowlands. In 2023, the field team observed a large fire that encroached into the forest and was halted only by a significant community firefighting effort and changing weather conditions. Ongoing efforts by Centre ValBio

and other partners aim to mitigate these risks through maintenance of firebreaks and provision of economic incentives for community-led conservation (Otero Jimenez et al., 2023).

Amphibians are especially vulnerable to fire and drought because of their physiological sensitivity to climate and dependence on water bodies for reproduction. Arboreal species may be particularly affected because the canopy dries faster than the forest floor, reducing available habitat and potentially forcing individuals to lower strata, where they may face greater competition or predation (Basham and Scheffers, 2020). In addition to effects of drying habitats, amphibians may face increasing risk from emerging pathogens such as *Bd*. Although we did not detect conclusive evidence of *Bd* at our study site, the IPA lies far outside the current monitoring sites used in Madagascar's National Monitoring Plan for *Bd* (Weldon et al., 2013). It is also located far from, and farther south than, any of the sites used to conduct herd immunity estimations for *Bd* based on cutaneous bacteria (Bletz et al., 2017). Given the likelihood of undescribed, range-restricted amphibians in the IPA, proactive monitoring remains essential to detect and mitigate potential disease impacts.

Whereas reptiles are generally more tolerant of heat and desiccation than amphibians, they remain highly vulnerable to habitat loss and land-use change in Madagascar. For instance, reptile richness has been shown to decline by over 50% in

burned forest areas in northeastern Madagascar (Fulgence et al., 2022), and more than 40% of Malagasy reptiles are currently considered to be threatened with extinction (Jenkins et al., 2014). In addition, Malagasy reptiles are heavily targeted for the international pet trade, particularly iconic species such as chameleons and geckos (Todd, 2011).

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LITERATURE CITED

- ANTONELLI, ALEXANDRE, RHIAN J. SMITH, ALLISON L. PERRIGO, ANGELICA CROTTINI, JAN HACKEL, WESTON TESTO, HARITH FAROOQ, MARIA F. TORRES JIMÉNEZ, NIELS ANDELA, TOBIAS ANDERMANN, ANDOTIANA M. ANDRIAMANOHERA, SYLVIE ANDRIAMBOLOLONERA, STEVEN P. BACHMAN, CHRISTINE D. BACON, WILLIAM J. BAKER, FRANCESCO BELLUARDO, CHRIS BIRKINSHAW, JAMES S. BORRELL, STUART CABLE, NATALY A. CANALES, JUAN D. CARRILLO, ROSIE CLEGG, COLIN CLUBBE, ROBERT S. C. COOKE, GABRIEL DAMASCO, SONIA DHANDA, DANIEL EDLER, SÖREN FAURBY, PAOLA DE LIMA FERREIRA, BRIAN L. FISHER, FÉLIX FOREST, LAUREN M. GARDINER, STEVEN M. GOODMAN, OLWEN M. GRACE, THAÍS B. GUEDES, MARIE C. HENNIGES, ROWENA HILL, CAROLINE E. R. LEHMANN, PORTER P. LOWRY, LOVANOMENJANAHARY MARLINE, PÁVEL MATOS-MARAVÍ, JUSTIN MOAT, BEATRIZ NEVES, MATHEUS G. C. NOGUEIRA, RENSKE E. ONSTEIN, ALEXANDER S. T. PAPADOPULOS, OSCAR A. PEREZ-ESCOBAR, LEANNE N. PHELPS, PETER B. PHILLIPSON, SAMUEL PIRONON, NATALIA A. S. PRZELOMSKA, MARINA RABERIMANARIVO, DAVID RABEHEVITRA, JEANNIE RAHARIMAMPIONONA, MAMY TIANA RAJAONAH, FANO RAJAONARY, LANDY R. RAJAONELONA, MIJORO RAKOTOARINIVO, AMÉDÉE A. RAKOTOARISOA, SOLOFO E. RAKOTOARISOA, HERIZO N. RAKOTOMALALA, FRANK RAKOTONASOLO, BERTHE A. RALAIVELOARISOA, MYRIAM RAMIREZ-HERRANZ, JEAN EMMANUEL N. RANDRIAMAMONJY, TIANJANAHARY RANDRIAMBOAVONJY, VONONA RANDRIANASOLO, ANDRIAMBOLOANTSOA RASOLOHERY, ANITRY N. RATSIFANDRIHAMANANA, NORO RAVOLOLOMANANA, VELOSOA RAZAFINIARY, HENINTSOA RAZANAJATOVO, ESTELLE RAZANATSOA, MALIN RIVERS, FERRAN SAYOL, DANIELE SILVESTRO, MARIA S. VORONTSOVA, KIM WALKER, BARNABY E. WALKER, PAUL WILKIN, JENNY WILLIAMS, THOMAS ZIEGLER, ALEXANDER ZIZKA, AND HÉLÈNE RALIMANANA. 2022. "Madagascar's Extraordinary Biodiversity: Evolution, Distribution, and Use." *Science* 378 (6623): eabf0869. <https://doi.org/10.1126/science.abf0869>.
- BARTYLAK, TOMASZ, PUSHPALATA KAYASTHA, MILENA ROSZKOWSKA, A. KEPEL, M. KEPEL, AND LUKASZ KACZMAREK. 2022. "Tardigrades of the Ivohibory Forest (South-Central Madagascar) with a Description of a New *Bryodelphax* Species." *European Zoological Journal* 89 (1): 423–36. <https://doi.org/10.1080/24750263.2022.2042404>.
- BASHAM, EDMUND W., MATTEO D. MASOTTI, BEATRIZ OTERO JIMENEZ, DENIS RAZAFITSIMALONA, GEORGES M. ANDRIANATENAINA, ANDOLALAO RAKOTOARISON, FRANK GLAW, PATRICIA WRIGHT, AND MIGUEL VENCES. 2026. "New Species of Treefrog of the *Boophis goudotii* Group from the Isolated Ivohiboro Protected Area in South-Eastern Madagascar." *Salamandra* 62 (1): 42–52.
- BASHAM, EDMUND W., AND BRETT R. SCHEFFERS. 2020. "Vertical Stratification Collapses Under Seasonal Shifts in Climate." *Journal of Biogeography* 47 (9): 1888–98. <https://doi.org/10.1111/jbi.13857>.
- BASHAM, EDMUND W., BRETT R. SCHEFFERS, AKIHIRO NAKAMURA, ABRAHAM BAMBA-KAYA, AND GREGORY F. M. JONGSMA. 2024. "Vertical Niche and Trait Associations in Central African Amphibians." *Biotropica* 56 (4): e13349. <https://doi.org/10.1111/btp.13349>.
- BASHAM, EDMUND W., CHRISTA M. SEIDL, LYDOU R. ANDRIAMAHOHATRA, BRUNNO F. OLIVEIRA, AND BRETT R. SCHEFFERS. 2019. "Distance-Decay Differs Among Vertical Strata in a Tropical Rainforest." *Journal of Animal Ecology* 88 (1): 114–24. <https://doi.org/10.1111/1365-2656.12902>.
- BELLUARDO, FRANCESCO, DARWIN DÍAZ QUIRÓS, JAVIER LOBÓN-ROVIRA, GONÇALO M. ROSA, MALALATIANA RASOAZANANY, FRANCO ANDREONE, AND ANGELICA CROTTINI. 2021. "Uncovering the Herpetological Diversity of Small Forest Fragments in South-Eastern Madagascar (Haute Matsiatra)." *Zoosystematics & Evolution* 97 (2): 315–43. <https://doi.org/10.3897/zse.97.63936>.
- BLETZ, MOLLY C., GONÇALO M. ROSA, FRANCO ANDREONE, ELODIE A. COURTOIS, DIRK S. SCHMELLER, NIRHY H. C. RABIBISOA, FALITIANA C. E. RABEMANANJARA, LILIANE RAHARIVOLONIAINA, MIGUEL VENCES, CHÉ WELDON, DEVIN EDMONDS, CHRISTOPHER J. RAXWORTHY, REID N. HARRIS, MATTHEW C. FISHER, AND ANGELICA CROTTINI. 2015. "Consistency of Published Results on the Pathogen *Batrachochytrium dendrobatidis* in Madagascar: Formal Comment on Kolby et al. Rapid Response to Evaluate the Presence of Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) and Ranavirus in Wild Amphibian Populations in Madagascar." *PLOS One* 10 (10): e0135900. <https://doi.org/10.1371/journal.pone.0135900>.
- BLETZ, MOLLY C., JILLIAN MYERS, DOUGLAS C. WOODHAMS, FALITIANA C. E. RABEMANANJARA, ANGELA RAKOTONIRINA, CHÉ WELDON, DEVIN EDMONDS, MIGUEL VENCES, AND REID N. HARRIS. 2017. "Estimating Herd Immunity to Amphibian Chytridiomycosis in Madagascar Based on the Defensive Function of Amphibian Skin Bacteria." *Frontiers in Microbiology* 8: 1751. <https://doi.org/10.3389/fmicb.2017.01751>.
- BLETZ, MOLLY C., ANGELICA CROTTINI, CHÉ WELDON, MIGUEL VENCES, FALITIANA C. E. RABEMANANJARA, M. FISHER, AND LOUIS DU PREEZ. 2022. "Amphibian Diseases and Parasites." In *The New Natural History of Madagascar*, edited by STEVEN M. GOODMAN, 1342–9. Princeton University Press. <https://doi.org/10.2307/j.ctv2ks6tbb.183>.
- BOYLE, DONNA G., DAVID B. BOYLE, VERONICA OLSEN, JESS A. T. MORGAN, AND ALEX D. HYATT. 2004. "Rapid Quantitative Detection of Chytridiomycosis (*Batrachochytrium dendrobatidis*) in Amphibian Samples Using Real-Time TaqMan PCR Assay." *Diseases of Aquatic Organisms* 60 (2): 141–8. <https://doi.org/10.3354/dao060141>.
- BROWN, JASON L., NEFTALI SILLERO, FRANK GLAW, PARFAIT BORA, DAVID R. VIEITES, AND MIGUEL VENCES. 2016. "Spatial Biodiversity Patterns of Madagascar's Amphibians and Reptiles." *PLOS One* 11 (1): e0144076. <https://doi.org/10.1371/journal.pone.0144076>.
- CHADWICK, ROBIN, PETER GOOD, GILL MARTIN, AND DAVID P. ROWELL. 2016. "Large Rainfall Changes Consistently Projected over Substantial Areas of Tropical Land." *Nature Climate Change* 6 (2): 177–81. <https://doi.org/10.1038/nclimate2805>.
- CHAN, KIN ONN, STEFAN T. HERTWIG, DARIO N. NEOKLEOUS, JANA M. FLURY, AND RAFE M. BROWN. 2022. "Widely Used, Short 16S rRNA Mitochondrial Gene Fragments Yield Poor and Erratic Results in Phylogenetic Estimation and Species Delimitation of Amphibians." *BMC Ecology & Evolution* 22 (1): 37. <https://doi.org/10.1186/s12862-022-01994-y>.
- DESBUREAUX, SÉBASTIEN, AND RICHARD DAMANIA. 2018. "Rain, Forests and Farmers: Evidence of Drought Induced Deforestation in Madagascar and Its Consequences for Biodiversity Conservation." *Biological Conservation* 221: 357–64. <https://doi.org/10.1016/j.biocon.2018.03.005>.
- FAHMY, MAI, DINA ANDRIANOELY, PATRICIA C. WRIGHT, AND EVON HEKKALA. 2023. "Leech-Derived iDNA Complements Traditional Surveying Methods, Enhancing Species Detections for Rapid Biodiversity Sampling in the Tropics." *Environmental DNA* 5 (6): 1557–73. <https://doi.org/10.1002/edn3.474>.
- FENG, XUE, AMILCARE PORPORATO, AND IGNACIO RODRIGUEZ-ITURBE. 2013. "Changes in Rainfall Seasonality in the Tropics." *Nature Climate Change* 3 (9): 811–15. <https://doi.org/10.1038/nclimate1907>.
- FISHER, MATTHEW C., AND TRENTON W. J. GARNER. 2020. "Chytrid Fungi and Global Amphibian Declines." *Nature Reviews. Microbiology* 18 (6): 332–43. <https://doi.org/10.1038/s41579-020-0335-x>.
- FRAPPIER-BRINTON, TRISTAN, AND SHAWN M. LEHMAN. 2022. "The Burning Island: Spatiotemporal Patterns of Fire Occurrence in

- Madagascar." *PLOS One* 17 (3): e0263313. <https://doi.org/10.1371/journal.pone.0263313>.
- FULGENCE, THIO ROSIN, DOMINIC A. MARTIN, R. RANDRIAMANANTENA, R. BOTRA, E. BEFIDIMANANA, KRISTINA OSEN, ANEEMARIE WURZ, HOLGER KREFT, ARISTIDE ANDRIANARIMISA, AND FANOMEZANA MIHAJA RATSOAVINA. 2022. "Differential Responses of Amphibians and Reptiles to Land-Use Change in the Biodiversity Hotspot of North-Eastern Madagascar." *Animal Conservation* 25 (4): 492–507. <https://doi.org/10.1111/acv.12760>.
- GLAW, FRANK, AND MIGUEL VENCES. 2007. *A Field Guide to the Amphibians and Reptiles of Madagascar*. 3rd ed. Vences and Glaw Verlag.
- GLAW, FRANK, MIGUEL VENCES, AND CHRISTOPHER JOHN RAXWORTHY. 2022. "Diversity and Exploration of the Malagasy Reptile Fauna." In *The New Natural History of Madagascar*, edited by STEVEN M. GOODMAN, 1423–42. Princeton University Press. <https://doi.org/10.2307/j.ctv2ks6tbb.203>.
- GONZALEZ, JUAN CARLOS T., ANNA PAULINE O. DE GUIA, JUDELIN C. DIMALIBOT, KHRYS V. PANTUA, WHIZVIR O. GUSTILO, AND NATHANIEL C. BANTAYAN. 2020. "Understorey to Canopy Vertebrate Fauna of a Lowland Evergreen Forest in Mt. Makiling Forest Reserve, Philippines." *Biodiversity Data Journal* 8: e56999. <https://doi.org/10.3897/bdj.8.e56999>.
- HARPER, GRADY J., MARC K. STEININGER, COMPTON J. TUCKER, DANIEL JUHN, AND FRANK HAWKINS. 2007. "Fifty Years of Deforestation and Forest Fragmentation in Madagascar." *Environmental Conservation* 34 (4): 325–33. <https://doi.org/10.1017/S0376892907004262>.
- HUTTER, CARL R., SHEA M. LAMBERT, ZO F. ANDRIAMPENOMANANA, FRANK GLAW, AND MIGUEL VENCES. 2018. "Molecular Phylogeny and Diversification of Malagasy Bright-Eyed Tree Frogs (Mantellidae: *Boophis*)." *Molecular Phylogenetics & Evolution* 127: 568–78. <https://doi.org/10.1016/j.ympev.2018.05.027>.
- HUTTER, CARL R., ZO F. ANDRIAMPENOMANANA, EDMUND W. BASHAM, FRANK GLAW, MATTEO D. MASOTTI, SHEA M. LAMBERT, AND MIGUEL VENCES. 2025. "A New Species of Mantellid Frog of the Genus *Guibemantis* from South-Eastern Madagascar." *Zootaxa* 5647 (3): 260–74. <https://doi.org/10.11646/zootaxa.5647.3.3>.
- IPCC (INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE). 2014. "2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change" *Climate Change*. <https://archive.ipcc.ch/report/ar5/syr/>.
- JENKINS, RICHARD K. B., MARCELO F. TOGNELLI, PHILIP BOWLES, NEIL COX, JASON L. BROWN, LAUREN CHAN, FRANCO ANDREONE, ALAIN ANDRIAMAZAVA, RAPHALI R. ANDRIANTSIMANARILAFY, MIRANA ANJERINAINA, PARFAIT BORA, LEE D. BRADY, ELISOA F. HANTALALAINA, FRANK GLAW, RICHARD A. GRIFFITHS, CRAIG HILTON-TAYLOR, MICHAEL HOFFMANN, VINEET KATARIYA, NIRHY H. RABISOA, JEANNOT RAFANOMEZANTSOA, DOMOINA RAKOTOMALALA, HERY RAKOTONDRAVONY, N. A. RAKOTONDRAZAFY, JOHANS RALAMBONIRAINY, JEAN-BAPTISTE RAMANAMANJATO, HERILALA RANDRIAMAHAZO, J. CHRISTIAN RANDRIANATOANDRO, HARISON H. RANDRIANASOLO, JASMIN E. RANDRIANIRINA, HIARINIRINA RANDRIANZAHANA, ACHILLE P. RASELIMANANA, ANDRIAMBOLANTSOA RASOLOHERY, FANOMEZANA M. RATSOAVINA, CHRISTOPHER J. RAXWORTHY, ERIC ROBSOMANITRANDRASANA, FINOANA ROLLANDE, PETER P. VAN DIJK, ANNE D. YODER, AND MIGUEL VENCES. 2014. "Extinction Risks and the Conservation of Madagascar's Reptiles." *PLOS One* 9 (8): e0100173. <https://doi.org/10.1371/journal.pone.0100173>.
- KATO, KAZUTAKA, AND DARON M. STANDLEY. 2013. "MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability." *Molecular Biology Evolution* 30 (4): 772–80. <https://doi.org/10.1093/molbev/mst010>.
- KAYS, ROLAND, AND ALLEN ALLISON. 2001. "Arboreal Tropical Forest Vertebrates: Current Knowledge and Research Trends." In *Tropical Forest Canopies: Ecology and Management*, edited by K. EDUARD LINSSENMAIR, ADRIAN J. DAVIS, BIRGIT FIALA and MARTIN R. SPEIGHT, 109–20. Springer. https://doi.org/10.1007/978-94-017-3606-0_9.
- KOLBY, JONATHAN E., KRISTINE M. SMITH, SARA D. RAMIREZ, FALITIANA RABEMANANJARA, ALLAN P. PESSIER, JESSE L. BRUNNER, CAREN S. GOLDBERG, LEE BERGER, AND LEE F. SKERRATT. 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): e0125330. <https://doi.org/10.1371/journal.pone.0125330>.
- KOLBY, JONATHAN E., AND LEE F. SKERRATT. 2015. "Amphibian Chytrid Fungus in Madagascar Neither Shows Widespread Presence nor Signs of Certain Establishment." *PLOS One* 10 (10): e0139172. <https://doi.org/10.1371/journal.pone.0139172>.
- KUMAR, SUDHIR, GLEN STECHER, AND KOICHIRO TAMURA. 2016. "MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets." *Molecular Biology Evolution* 33 (7): 1870–4. <https://doi.org/10.1093/molbev/msw054>.
- LAMBERT, MAX R., MOLLY C. WOMACK, ALLISON Q. BYRNE, OBED HERNÁNDEZ-GÓMEZ, CLAY F. NOSS, ANDREW P. ROTHSTEIN, DAVID C. BLACKBURN, JAMES P. COLLINS, MARTHA L. CRUMP, MICHELLE S. KOO, PRIYA NANJAPPA, LOUISE ROLLINS-SMITH, VANCE T. VREDENBURG, AND ERICA B. ROSENBLUM. 2020. "Comment on 'Amphibian Fungal Panzootic Causes Catastrophic and Ongoing Loss of Biodiversity.'" *Science* 367 (6484): eaay1838. <https://doi.org/10.1126/science.aay1838>.
- LIPS, KAREN R. 2016. "Overview of Chytrid Emergence and Impacts on Amphibians." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 371 (1709): 20150465. <https://doi.org/10.1098/rstb.2015.0465>.
- MIRALLES, AURÉLIEN, JÖRN KÖHLER, FRANK GLAW, KATHARINA C. WOLLENBERG VALERO, ANGELICA CROTTINI, GONÇALO M. ROSA, LOUIS DU PREEZ, PHILIP-SEBASTIAN GEHRING, DAVID R. VIEITES, FANOMEZANA M. RATSOAVINA, AND MIGUEL VENCES. 2023. "An Endless Harvest: Integrative Revision of the *Gephyromantis boulengeri* and *G. blanci* Complexes Reveals Six New Species of Mantellid Frogs from Madagascar." *Salamanca* 59 (1): 1–41.
- MYERS, NORMAN, RUSSELL A. MITTERMEIER, CRISTINA G. MITTERMEIER, GUSTAVO A. B. DA FONSECA, AND JENNIFER KENT. 2000. "Biodiversity Hotspots for Conservation Priorities." *Nature* 403 (6772): 853–58. <https://doi.org/10.1038/35002501>.
- NEELIN, J. DAVID, MATTHIAS MÜNNICH, H. SU, JOYCE E. MEYERSON, AND C. E. HOLLOWAY. 2006. "Tropical Drying Trends in Global Warming Models and Observations." *Proceedings of the National Academy of Sciences of the United States of America* 103 (16): 6110–15. <https://doi.org/10.1073/pnas.0601798103>.
- NORMAN, MYERS. 2003. "Biodiversity Hotspots Revisited." *BioScience* 53 (10): 916–7. [https://doi.org/10.1641/0006-3568\(2003\)053\[0916:BHR\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2003)053[0916:BHR]2.0.CO;2).
- OTERO JIMENEZ, BEATRIZ, REN MONTAÑO, RYAN S. ROTHMAN, RACHEL C. WILLIAMS, AND PATRICIA C. WRIGHT. 2023. "A Surprising Haven: The Biodiversity of an Old-Growth Forest Amidst a Scorched Landscape in Madagascar." *Conservation Science & Practice* 5 (9): e12993. <https://doi.org/10.1111/csp2.12993>.
- PALUMBI, STEPHEN R., ANDREW MARTIN, SANDRA ROMANO, W. OWEN MCMILLAN, LIGAYA STICE, AND GAIL GRABOWSKI. 1991. *The Simple Fool's Guide to PCR. version 2.0*. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.
- R CORE TEAM. 2025. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. <https://www.R-project.org/>.
- RIGDEN, ANGELA, CHRISTOPHER GOLDEN, DUO CHAN, AND PETER HUYBERS. 2024. "Climate Change Linked to Drought in Southern Madagascar." *npj Climate & Atmospheric Science* 7 (1). <https://doi.org/10.1038/s41612-024-00583-8>.
- SCHEELE, BEN C., FRANK PASMANS, LEE F. SKERRATT, AND LEE BERGER, AN MARTEL, WOUTER BEUKEMA, ALDEMAR A. ACEVEDO, PATRICIA A. BURROWS, TAMILIE CARVALHO, ALESSANDRO CATENAZZI, IGNACIO DE LA RIVA, MATTHEW C. FISHER, SANDRA V. FLECHAS, CLAIRE N. FOSTER, PATRICIA FRÍAS-ÁLVAREZ, TRENTON W. J. GARNER, BRIAN GRATWICKE, JUAN M. GUAYASAMIN, MAREIKE HIRSCHFELD, JONATHAN E. KOLBY, TIFFANY A. KOSCH, ENRIQUE LA MARCA, DAVID B. LINDENMAYER, KAREN R. LIPS, ANA V. LONGO, RAÚL MANEYRO, CAIT A. McDONALD, JOSEPH MENDELSON III, PABLO PALACIOS-RODRIGUEZ, GABRIELA PARRA-OLEA, CORINNE L. RICHARDS-ZAWACKI, MARK-OLIVER RÖDEL, SEAN M. ROVITO, CLAUDIO SOTO-AZAT, LUÍS FELIPE TOLEDO, JAMIE VOYLES, CHÉ WELDON, STEVEN M. WHITFIELD, MARK WILKINSON, KELLY R. ZAMUDIO, AND STEFANO CANESSA. 2019. "Amphibian Fungal Panzootic Causes Catastrophic and Ongoing Loss of Biodiversity." *Science* 363: 1459–63. <https://www.undp.org/belarus/publications/next-frontier-human-development-and-anthropocene>.
- SCHEFFERS, BRETT R., REBECCA M. BRUNNER, SARA D. RAMIREZ, LUKE P. SHOO, ARVIN DIESMOS, AND STEPHEN E. WILLIAMS. 2013. "Thermal Buffering of Microhabitats Is a Critical Factor Mediating Warming Vulnerability of Frogs in the Philippine Biodiversity Hotspot." *Biotropica* 45 (5): 628–35. <https://doi.org/10.1111/btp.12042>.
- STEIGERWALD, EMMA, JULIA CHEN, JULIANNE OSHIRO, VANCE T. VREDENBURG, ALESSANDRO CATENAZZI, AND MICHELLE S. KOO. 2024. "Microreserves Are an Important Tool for Amphibian Conservation." *Communications Biology* 7 (1): 1177. <https://doi.org/10.1038/s42003-024-06510-0>.

- STOREY, MARTIN, JOHN J. MAHONEY, ANDREW D. SAUNDERS, ROBERT A. DUNCAN, SIMON P. KELLEY, AND MILLARD F. COFFIN. 1995. "Timing of Hot Spot-Related Volcanism and the Breakup of Madagascar and India." *Science* 267 (5199): 852–5. <https://doi.org/10.1126/science.267.5199.852>.
- TODD, MATTHEW. 2011. *Trade in Malagasy Reptiles and Amphibians in Thailand*. TRAFFIC Southeast Asia Report.
- UNITED NATIONS DEVELOPMENT PROGRAMME. 2023. *Human Development Report 2023: The Next Frontier—Human Development and the Anthropocene*. United Nations. Available at: <https://hdr.undp.org/en>.
- VENCES, MIGUEL, STEFANOS PATMANIDIS, VLADIMIR KHARCHEV, AND SUSANNE S. RENNER. 2022. "Concatenator, a User-Friendly Program to Concatenate DNA Sequences, Implementing Graphical User Interfaces for MAFFT and FastTree." *Bioinformatics Advances* 2 (1): vbac050. <https://doi.org/10.1093/bioadv/vbac050>.
- VIEITES, DAVID R., KATHARINA C. WOLLENBERG, FRANCO ANDREONE, JÖRN KÖHLER, FRANK GLAW, AND MIGUEL VENCES. 2009. "Vast Underestimation of Madagascar's Biodiversity Evidenced by an Integrative Amphibian Inventory." *Proceedings of the National Academy of Sciences of the United States of America* 106 (20): 8267–72. <https://doi.org/10.1073/pnas.0810821106>.
- VONESH, JAMES R., JOSEPH C. MITCHELL, KIM HOWELL, AND ANDREW J. CRAWFORD. 2009. "Rapid Assessments of Amphibian Diversity." In *Amphibian Ecology and Conservation: A Handbook of Techniques*, edited by C. KENNETH DODD JR., 263–80. Oxford University Press. <https://doi.org/10.1093/oso/9780199541188.003.0015>.
- WELDON, CHÉ, ANGELICA CROTTINI, AN BOLLEN, FALITIANA C. E. RABEMANANJARA, JAMIE COPSEY, GERARDO GARCIA, AND FRANCO ANDREONE. 2013. "Pre-emptive National Monitoring Plan for Detecting the Amphibian Chytrid Fungus in Madagascar." *EcoHealth* 10 (3): 234–40. <https://doi.org/10.1007/s10393-013-0869-8>.
- WICKHAM, HADLEY. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag. <https://ggplot2.tidyverse.org>.
- WILMÉ, LUCIENNE, STEVEN M. GOODMAN, AND JÖRG U. GANZHORN. 2006. "Biogeographic Evolution of Madagascar's Microendemic Biota." *Science* 312 (5776): 1063–5. <https://doi.org/10.1126/science.1122806>.

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