

Expedition Angano

Report to Funders and Stakeholders



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Executive Summary

Madagascar possesses unparalleled levels of biological diversity and endemism. Habitat loss and forest fragmentation pose a significant threat to its native flora and fauna. Recent research estimates that globally, 70% of forest is within 1 km of a non-forest edge. The proportion of remaining forest that persists in small relict fragments with high edge ratios is increasing in Madagascar. In December 2015 we led an international research expedition to the commune of Beandrazona in the Sofia province of northern Madagascar, to assess the impact of altered habitat conditions at forest edges on endemic herpetofauna.

We built upon previous inventory-based research by investigating edge effects in the forest fragments between the protected areas of Bemanavika and Tsaratanana, within a proposed protection corridor that has so far been largely unstudied. Using reptiles and amphibians as indicator species, we employed standard ecological monitoring methods (transects, active searching, acoustic monitoring, and pitfall traps) to assess the diversity of herpetofauna and their distribution in relation to forest edges.

Our research enabled us to identify several new species to science, discover additional specimens of previously poorly known species, and expand the distribution of several known species. Our data will also directly contribute to the IUCN Red List assessment of several species. Furthermore, we identified characteristic edge-avoiding or interior-avoiding responses in several species groups, and found evidence that, contrary to expectations, many effects of edges appear to dissipate after ~30 m into the forest. With several newly proposed protected areas close to our research site, we anticipate that this research will be highly relevant to conservation practitioners assessing the conservation value of small and medium sized forest fragments.

Key to the expedition was close collaboration between international and Malagasy researchers before, during and post-expedition, and the support of numerous individuals and organisations. Several peer-reviewed publications will be produced from the results of this expedition.



A sample of frog species recorded from Ampotsidy. (Photographs by Mark D. Scherz)

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Acknowledgements

The expedition gratefully acknowledges the generosity of the numerous individuals and organisations that supported Expedition Angano. In particular, we thank Sally Reid, Nisha Owen, Shane Winser, Huw Jones, Nathalie Evans and Charlotte Cole for supporting grant applications. Peter Long, Richard Jenkins, Craig Turner, Rob Gandola, Nigel Winser, Lance Woolaver, Steve Le Comber and Rob Knell for useful advice and discussions on methodologies. Frank Glaw, Miguel Vences, Angelica Crottini, and Jörn Köhler for advice on taxonomic issues, and Ella Z. Lattenkamp for help in preparation of sampling resources. We thank Sylvia Knight for generously trusting us with meteorological equipment, colleagues at QMUL for permitting use of ibutton dataloggers, Lloyd Figgins and Tino Solomon for risk and medical advice and Jon Grey for references. We are extremely grateful to Brian Fisher and the staff of the Madagascar Biodiversity Center in Antananarivo for their help and advice, and for the loan of collection bags and a generator.

In addition to grant funding, we are greatly indebted to the 50 individuals who donated a total of £1,410 to our expedition through Indiegogo—their generosity made a great deal of our research possible.

Finally, we would particularly like to thank the staff of MICET for handling our permits, vehicles, and some tricky logistical issues. In this vein, we thank our drivers, guides and cooks, Davy and Jean-Claude, Jean-René, Colonel, Vilno, Serge, and Soa as well as the people of Beandrarezona, without whom we would not have been able to work in such an incredible location.



A team of porters from Beandrarezona en route to basecamp with food supplies.

1. Expedition Field Site and Logistics

The expedition aimed to conduct research in typical fragmented mid-altitude rainforest. A small cluster of mountains was chosen, known locally as Ampotsidy. These lie almost due north of Bealanana, close to the village of Beandrarezona. The study area was selected due to the close proximity of several newly designated protected areas and forest corridors. Evidence of slash-and-burn agriculture (or *tavy*) was noticeable in several localities in the research area. Parts of the team departed separately from Antananarivo and Antsiranana, rendezvoused in Antsohihy, and then travelled via Bealanana to the small village of Beandrarezona. The journey involved three 4x4 vehicles, several zebu carts, and then eight kilometres by foot.

The expedition was during the wet season, from the 10th December 2015, to the 16th January 2016, permitting approximately four weeks of research. Three guides, two cooks and approximately 30 porters were employed from Beandrarezona. Basecamp was established at 14.4191°S, 48.7190°E, on the western forested slope of the central Ampotsidy peak. In this area, extremely well-defined edges are formed between structurally intact, though partly degraded primary forest, and extensive open shrubland. The forests under study ranged in elevation between 1100 and 1550 m a.s.l. and showed characteristic mid- and high-altitude rainforest. This is a particularly wet region of Madagascar, with January being the wettest month averaging 330 mm precipitation (climate-data.org). Fragment size varied from small riverine forest strips to large stretches of forest which are in fact continuous with the large forest belt that extends more or less continuously from Tsaratanana in the west to Makira in the east. Non-forest habitats included marshy grassland (possibly former rice paddy), exposed savannah grassland and shrubland, and localised areas of cultivated land, growing primarily bananas but also other crops.

Landsat products are frequently used in monitoring and assessments of land-cover, land-cover change, and ecosystem dynamics such as disturbance and vegetation health, both at local and global scales. The multispectral scanners onboard the Landsat satellites detect reflected light from plant chlorophyll, which can be easily differentiated from more impervious surfaces through the red and near-infrared wavelengths. Landsat products for the study region were obtained through the Global Land Survey (GLS) datasets (GLS 2005, GLS 2000, GLS 1990, and GLS 1975) created by USGS and NASA. Based on visual interpretation of the images, the forest edge along which the expedition conducted its transects dates back to at least 1975, which coincides with the launch of the Landsat 2 satellite. Images prior to 1975 are not available for our study area and it is therefore impossible to tell with remote sensed data how old the forest edges are, although we can state with confidence that they are at least forty years old.



Utilising extensive local knowledge to identify a suitable basecamp location



Over the course of the expedition more than 30 local people were employed. Here, porters have arrived at basecamp with supplies.



Figure 1. Map of Madagascar depicting the main provinces, the capital city and the expedition base camp.

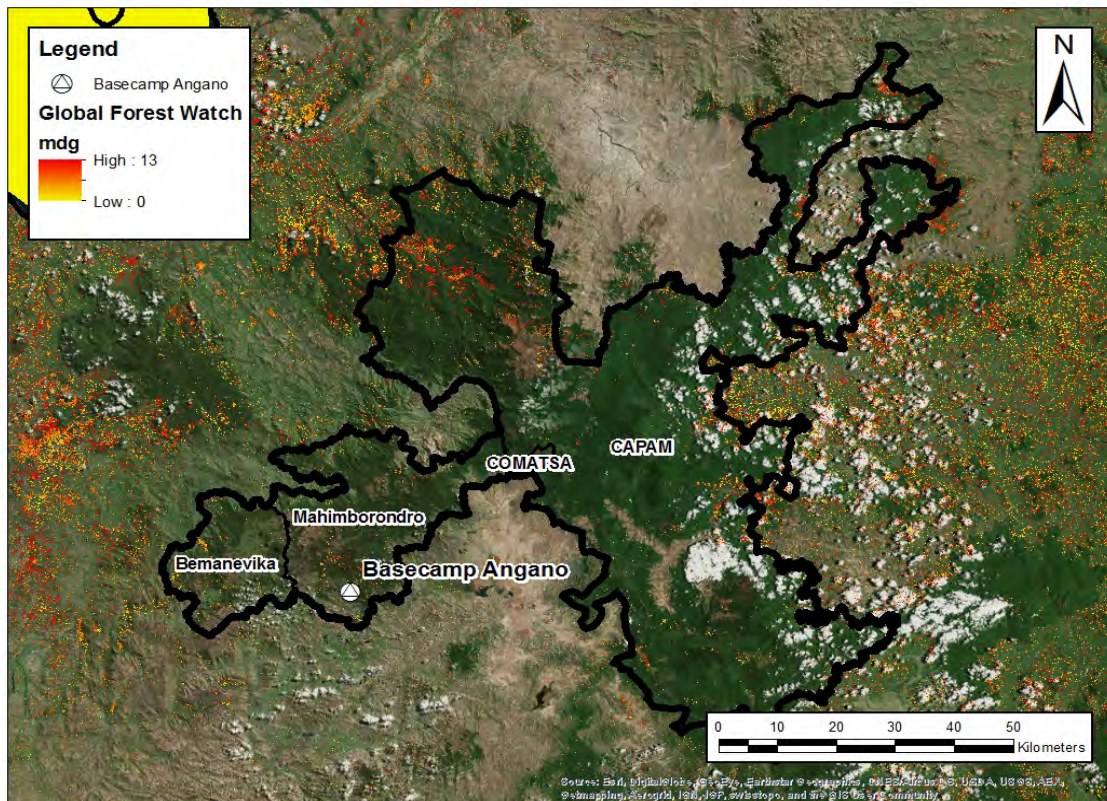


Figure 2. CAPAM protected areas with Global Forest Watch deforestation data superimposed

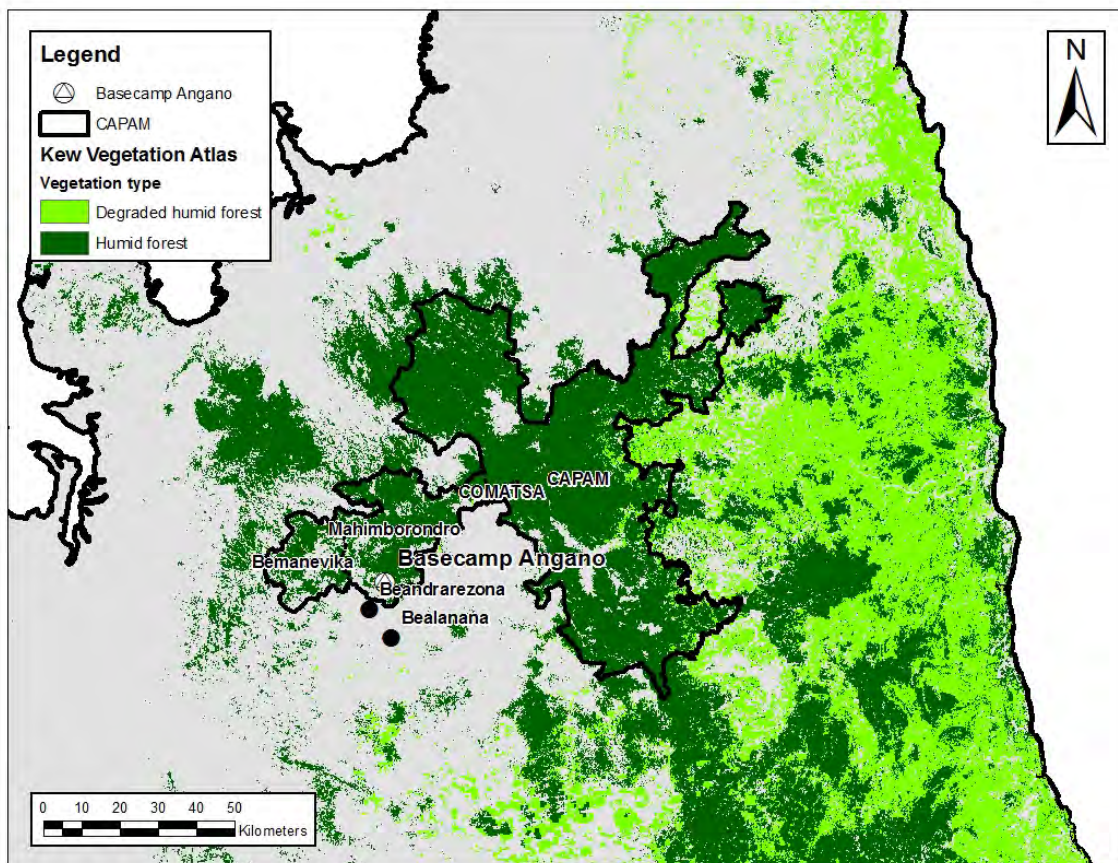


Figure 3. CAPAM protected areas and forest cover from the Kew Vegetation Atlas of Madagascar

1.2 Images of local area



Dawn over mid-altitude primary rainforest near to basecamp



Image of our study region, showing clearly defined forest edge

2. Expedition Team

The expedition team consisted of international researchers from the UK and Germany, together with scientists affiliated with two Malagasy Universities, as well as local NGOs.



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Herpetologist
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GIS and Data Specialist
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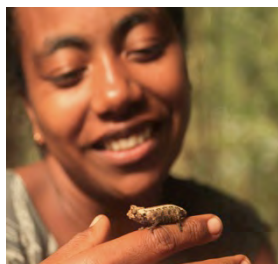
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3. Introduction to Research

Natural expanses of tropical forest usually encompass a dynamic mosaic of habitat types (Whitmore 1998). These may include clearings and subtle vegetation transitions as a result of treefall, succession, abiotic landscape features and other disturbance regimes (Chazdon 2003; Pickett & White 2013). However, contemporary anthropogenic pressures have led to a reorganisation of tropical forest structure and dynamics (Wright 2005), with approximately half of potential closed-canopy forest removed and the remainder increasingly fragmented and degraded (Keenan et al. 2015; Riitters et al. 2000; Wade et al. 2003; Wright 2005). As a consequence, a global forest cover analysis estimated that 70% of remaining forest is within 1 km of the forest's edge (Haddad et al. 2015).

Edges are characterised by changes in habitat type, such as the transition of rainforest to pasture, and expose organisms in the forest fragment to the prevailing conditions of the surrounding ecosystem (Murcia 1995). In the Brazilian Amazon it has been estimated that deforestation results in 20,000 km of new forest edge each year (Giles & Burgoyne 2008), though estimates are sparse for other regions. The interaction between relict adjacent habitat types and the subsequent changes in biotic and abiotic conditions has been termed 'edge effects' (Saunders et al. 1991). In the context of rainforest fragments, edge effects may modify the forest conditions beyond the range of natural variation, rendering the edge unsuitable for inhabitants of the original, deep forest. Thus the area that contributes to conservation may be less than the total fragment size. This is confounded by the paradox that habitat complexity at edges often results in higher species diversity, yet with interior species lacking.

Numerous studies have attempted to quantify edge effects and the response of rainforest plant and animal communities (reviewed in Murcia 1995). Abiotic effects close to forest edges have been found to include increased solar radiation and wind speed, reduced air and soil moisture, higher temperatures and wider temperature fluctuations (Camargo & Kapos 1995; Davies-Colley et al. 2000; Matlack 1993). This may result in altered microclimatic gradients and an impaired ability to buffer daily abiotic cycles (Ewers & Banks-Leite 2013). In response, forest edges experience higher veteran tree mortality (Laurance et al. 2000), altered vegetation structure (Didham & Lawton 1999; Harper et al. 2005; Matlack 1994), leaf litter depth (Didham & Lawton 1999), seed dispersal (Cadenasso & Pickett 2001), predation (Vetter et al. 2013), and, somewhat counter intuitively, broader species richness and composition (Laurance et al. 2007). Indeed in some cases forest edges gradually recede due to turnover in plant communities, thereby autonomously reducing the size of fragments until the original habitat ultimately disappears (Gascon et al. 2000).

Despite this extensive field of research, a consensus on the depth at which 'edge effects' penetrate (and indeed how this varies across regions and habitats) has not been reached. Estimates for different abiotic factors vary from as little as 20 m (Ewers & Banks-Leite 2013) to more than 1 km (Gascon et al. 2000; Laurance 2004) with the majority diminishing by around 50 m into the forest (Murcia 1995; Whitmore et al. 1997). The response of wild populations to edge effects is only known for a limited number of species groups (Ries et al. 2004; Fahrig 2003).

Madagascar is home to some of the most biologically rich forests in the world (Myers et al. 2000), yet has also experienced some of the most extensive deforestation, with approximately 40% forest cover loss since the 1950s (Harper et al. 2007). The same study found that the percentage of remaining forest within 0–57 m of a forest edge had increased from 3.6% to 25.1% between the 1950s and c. 2000. Unlike Brazil and other countries with tropical forests, Madagascar's deforestation is largely civil and not commercial, driven by subsistence agriculture, and not by the wood or mining industry

(with rare exceptions). Conservation of its forests is correspondingly more difficult than simple injunctions against industries, but instead requires community-based interventions that provide education and alternative livelihood options. Numerous studies have investigated species composition in surviving fragmented and/or degraded forests, frequently finding declines in biodiversity (Rakotondravony & Goodman 2011; Irwin et al. 2010; Riemann et al. 2015; Jenkins et al. 2003). However, few have explicitly considered the contribution of edge effects to these processes (Lehtinen et al. 2003; Ramanamanjato & Lehtinen 2006; Lehman et al. 2006).

In this study we conduct the first assessment of edge effects in typical mid- to high-altitude rainforest of Northern Madagascar using reptiles and amphibians as indicators. These taxa in particular are strongly dependent on the presence of suitable microhabitats to meet physiological needs (e.g. reproduction, thermoregulation), and their distributions are thus likely to respond to edges (Lehtinen et al. 2003). Madagascar has exceptionally high levels of herpetofaunal diversity, with 99% (non-introduced) amphibian and 92% reptile endemism (Glaw & Vences 2003, 2007). Most of these species are in fact microendemic, with small ranges of specific localised habitats (Brown et al. 2014, 2016; Wollenberg et al. 2008, 2011, 2012). This diversity, encompassing over 700 described and an estimated >300 undescribed taxa (Vieites et al. 2009; Perl et al. 2014; Nagy et al. 2012), provides an ideal system in which to investigate the variety of possible species responses to forest edges.



Deforested landscape to the south of our research site (Photo by D. Parker)

3.1 Research Questions

1) At what distance from the forest edge can we detect altered community composition in herpetofauna?

2) Do responses to forest edges differ between species and across taxonomic groups?

3) Which species are present on the Ampotsidy mountains, and how do edge effects influence their conservation status?

3.2 Additional outcomes

In addition to addressing the questions posed above, the expedition had a number of broader aims to support research and conservation in Madagascar.

- To support the training and development of Malagasy graduate students, through participation in Expedition Angano. To achieve this, we funded three students' involvement and supplied data for two Masters research projects.
- To engage with the local community, and support and promote sustainable development.
- To develop a short film focusing on the work undertaken by the expedition to bring the concepts of field research, edge effects and wildlife conservation to a broader audience.
- To deposit specimens at the Université d'Antananarivo Département Biologie Animale and Zoologische Staatssammlung München. A small set of specimens were also collected to assist in the formation of a scientific research collection at the Université d'Antsiranana.

4. Research Materials and Methods

4.1 Transects

A total of 24 transects were established, oriented perpendicular to the forest edge. Of these, 22 were 120 m in length and two were shorter due to small fragment size. Transects were designed to extend 100 m into the forest, and 20 m out from the forest edge into the surrounding non-forest matrix to adequately capture the transition in habitat type. The ‘zero metres’ point was defined as the first woody vegetation greater than 2m in height and with a diameter at breast height (dbh) >5 cm (as in Lehtinen et al. 2003). In total, 2790 m of transects were marked.

Approximate transect locations were dispersed across the study location, with a minimum of 100 m between each start point to maintain independence (Figure 1). Precise positions were then identified with a random number generator to avoid inherent biases such as selecting less dense forest. Direction accuracy was maintained using a compass bearing, and confirmed using GPS coordinates. Biodegradable flagging tape was positioned every 10 m to aid subsequent surveys. To minimise disturbance, transects were kept narrow, very little vegetation was cut, and at least 48 hours was maintained between setting transects and the first survey, with 72 hours or more separating subsequent surveys.

Between two and four participants searched each transect. A small number of pilot surveys were conducted with the whole team to ensure methods were consistent. Average survey length was 85 minutes. 102 transect replicates were performed (54 day, 48 night), totalling 352 person-hours of survey time.



Thom Starnes entering data between surveys. (Photo by D. Parker)

For each transect, the following information was recorded:

- A unique transect event number
- The transect name, replicate number and date
- Transect participants
- Start and end time
- Current weather conditions (Sunny, cloudy, clear, light rain, medium rain or heavy rain) and approximate time since last rainfall (raining, rained within 3/6/12/24/24+ hours).

Audio and visual detection, as well as non-destructive active searching within refugia (such as tree holes, deadwood, *Pandanus* leaf axils and leaf litter) were used to detect amphibians and reptiles. All specimens were determined as far as possible *in situ* using the Field Guide to the Amphibians and Reptiles of Madagascar 3rd Edition (Glaw & Vences 2007) and subsequently published species descriptions. Where identification was in doubt, or where the species was potentially new to the survey, specimens were returned in specimen bags for more detailed study at basecamp.

For each observation, the following information was recorded:

- A unique sample number (to be placed in the specimen bag if it was to be retained)
- Transect distance (distance from zero)
- Perpendicular distance from line
- Height
- Microhabitat notes
- In some cases, in-situ photographs and audio recordings were also made

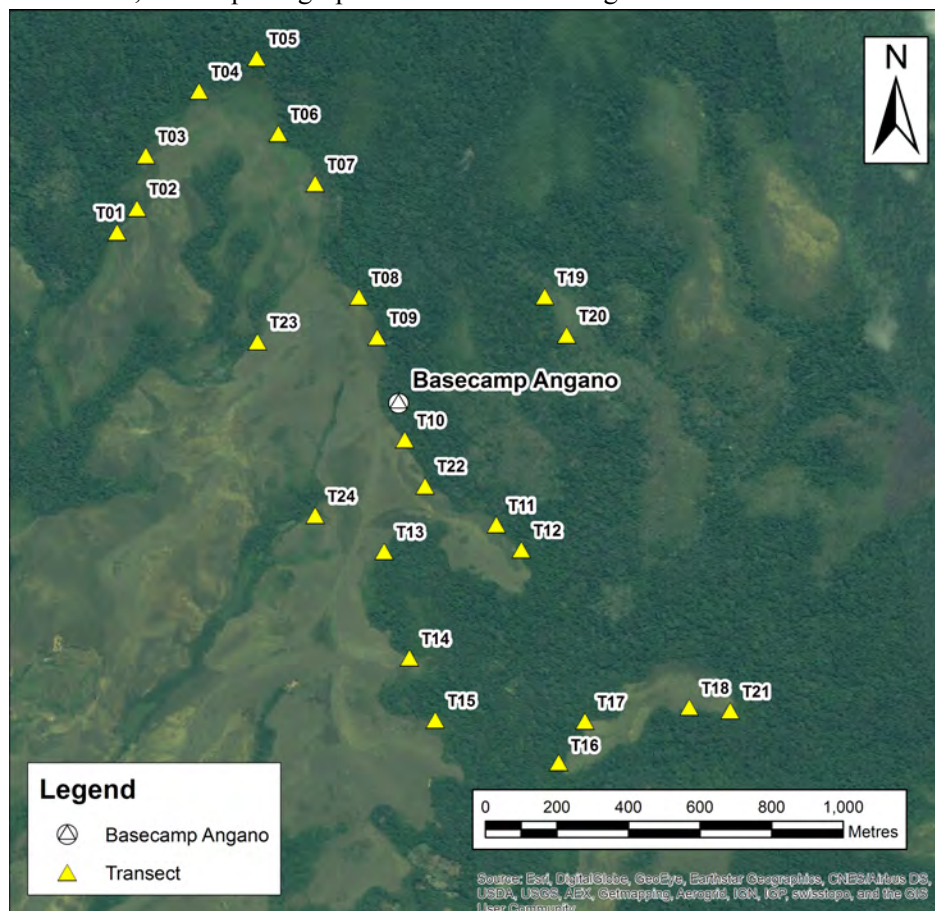


Figure 4. Location of transects and basecamp within the study region

4.2 Randomization analysis

Initial analysis broadly followed the randomization method of Lehtinen et al. (2003), with a null hypothesis predicting that each species is randomly distributed along edge-interior transects. Under this scenario, with sufficient samples, the mean detection distance within forest is expected to converge on 50 m. We excluded data from outside the forest (-20–0 m) from this analysis.

To test whether the data deviates from the null model, data were pooled into sub-genus level groupings (where sub-genera exist; where they do not, genus-level grouping was used) to maximise ecological similarity within groupings, with mean and standard deviation values calculated. An equivalent number of values from 0 to 100 were drawn at random and summed, over 1000 iterations, in order to generate a null distribution. The mean value from real data could then be compared to this null distribution. The number of times the null distribution is more extreme than the real data indicates the probability of observing this mean detection distance.

In this analysis we treat values <0.1 as significant interior avoiders, and values >0.9 as significant edge avoiders. This method generates a unique distribution based on the sample size for each sub-genus group, and thus is robust to relatively small sample sizes and is unlikely to suffer from type I errors. However we nonetheless suggestion caution in interpreting results from small sample groupings. A key difference to Lehtinen et al.'s (2003) method is the use of geometric means, which are more robust to outliers. Histograms of raw data were plotted, with geometric means and p-values drawn from the null distribution using custom scripts in R software (R Development Core Team 2014).

A potentially confounding factor in this analysis is the presence of small rainforest streams and rivers. Several amphibian species are dependent on streams for part or all of their lifecycle. In this study, care was taken to place transects such that the position of streams on edge-interior transects varied, but this was not always possible. To assess the impact of streams on species distribution data, mean stream position was calculated and positions plotted.

4.3 Distance analysis

An improved analysis method is currently being developed that will permit calculation of density across different distance classes. This should be a substantial improvement on previously published analyses and will be the subject of a peer-reviewed journal article.

4.4 Habitat Assessment

Whilst the main objective of the expedition was to utilise herpetofauna as indicators of edge effects, rapid habitat surveys were carried out at each transect to assess possible changes in the habitat due to edge effects and topography. Transects varied in elevation, micro-topography, habitat structure, hydrology, and disturbance levels. Several of the transects were intercepted by habitat features including streams, rocks and boulders, mature lianas, dense thickets, and fallen trees.

The habitats of all 24 transects were surveyed during daylight hours by just one team member in order to minimise bias, with occasional supervision by another member. A range of habitat measurements

were collected within 5 m x 5 m quadrats at four distances along the transect; -20 m, 5 m, 50 m and 100 m. Eleven measurements were chosen that could be collected rapidly by a single team member and were non-destructive, leaving negligible changes to the transect environments. Collectively, the measurements had to provide insights into the relationship between the vegetation components of the habitat and the distance to the forest edge. Table 1 summarises the measurements collected and provides justification for their inclusion.

One-way analysis of variance (ANOVA) tests were carried out on the data sets with numerical measures to determine if distance from the forest edge had a significant effect on each variable. Tukey's honest significance tests were used post-hoc to determine between which distances the results were significant. A chi squared test (with and without simulated p-values) and Fisher's exact test were applied to the categorical variable data of understory density and burning evidence.

Table 1. Habitat assessment characteristics that are considered relevant to edge response.

Measurement	Justification
Number of <i>Pandanus</i> heads	<i>Pandanus</i> are slow growing and appear to germinate poorly in degraded conditions. They are mostly associated with water features, including swamps and streams. They are also a valuable microhabitat for specialised herpetofauna (Lehtinen 2002).
Understory density	In tropical forests understory density usually decreases with increasing distance from the edge (Malcolm 1994; Murcia 1995).
Percentage Canopy Cover (>5 m height)	In tropical forests canopy cover usually increases with increasing distance from the edge (Malcolm 1994).
No. of tree >50mm diameter Circumference at breast height	Large mature trees indicate lower levels of disturbance and would be expected to be more abundant with increasing distance from the edge (Murcia 1995).
Burning	Evidence of burning provides information on the disturbance history of the transect. The effect of fires on forest edges differs depending on their structure (Jenkins et al. 2003).
Cut stumps	Cut stumps provide information on the disturbance history of the transect (Jenkins et al. 2003).
Bracken	Bracken (<i>Pteridium aquilinum</i>) stems were counted to assess the possibility that their abundance decreases with increasing distance into the forest.
Ground ferns	Ground and epiphytic ferns were counted to assess the possibility that their abundance increases with increasing distance into the forest and higher humidity levels (Vajda et al. 2001).
Diameter of dead-fall > 5 m length	The volume of substantial (>5 m length) deadfall was measured to observe potential variation in deadfall with distance from the edge.
Leaf litter depth	Leaf litter depth was measured to assess the possibility that it becomes deeper with increasing distance into the forest.

4.5 Microclimate

Ten iButton ThermoChron® (Maxim Integrated, San Jose, USA) temperature data loggers were deployed on a subset of 100 m forest transects at 10 m intervals from 0 m to 90 m. Temperature loggers were numbered with a permanent marker on electrical insulation tape and placed in a re-sealable watertight plastic bag with ca. 10 g of silica gel to absorb any ambient moisture from inside the bag. Bags were then attached to the boles of trees at breast height (ca. 1.4 m) out of direct sunlight, as close to the 10 m interval markers as practicable (± 1.5 m) with utility cord.

Temperature loggers were left in situ for a period of at least 36 hours beginning at 0000 h or 1200 h. This allows the temperature data to be compared in 24 hour periods from midday to midday or midnight to midnight. As it would always be necessary to transport the iButtons from one transect to another which could take anywhere between 30 minutes to 3 hours, it would not be possible to log consecutive 24 hour periods. The 36 hour time period was therefore deemed an appropriate way to maximise the volume of data collected. All times are given in local time (East Africa Time Zone = UTC + 03:00).

It was also necessary to periodically return all ten iButtons to basecamp in order to download the temperature data from the data loggers to the field computer, and wipe the data loggers' internal storage. The iButtons' internal memory capacity allowed for 2048 total temperature recordings to be logged. At a recording rate of 10-minute intervals, the memory needed to be formatted every 14 days. The iButton temperature loggers were connected to the field computer via a 1-wire USB interface, facilitating rapid data transfer.

Data analysis was conducted in R software (R Development Core Team 2014). Data from each distance class (e.g. 10 m, 20 m) was collated using the package *reshape2* and visualised using boxplots. Additional means were also calculated and plotted. Separately, the packages *zoo* and *ggplot2* (Wickham 2009) were used to plot a smoothed temperature traces for each transect individually over a 36 hour period.

4.6 Drone imagery

The expedition also explored the potential for habitat analysis using drone generated georeferenced orthomosaic maps of the study area. Preliminary results are presented, but this can be considered more a proof of concept than a coherent analysis.

4.7 Taxonomic research and additional surveys

In addition to our core survey strategy, pitfall trapping, targeted searching, and opportunistic encounters supplemented our research. This provided a more complete picture of the herpetofauna of Ampotsidy, and also allowed us to identify taxa missed by other methods.

A 50 m pitfall trapping line was established close to basecamp, running parallel to and approximately 100 m from the forest edge. The line consisted of a plastic sheeting drift fence, with buckets (20 litre) spaced every 10 metres (n=6) sunk to or below ground level. This was checked twice daily, or more frequently during heavy rain. Targeted searches focused on forest streams, springs, patches of

waterlogged grassland, large *Pandanus* stands, and rocky outcrops that were missed in transects. When possible, calling male frogs were recorded on a Marantz PMD661 MKII with a Sennheiser ME66 super-cardioid microphone before capture.

Specimens found during transects or other surveys that could not be identified in the field, or that represented potentially new taxa for our study, were captured and brought to camp for identification. Vouchers of each species were collected for taxonomic and species record purposes, following standard field procedures and ethics guidelines: frogs were euthanized by immersion in a solution of 0.5% MS222; reptiles were euthanized by intracardiac injection of concentrated MS222. A tissue sample was taken from these animals and placed in 99% ethanol for later DNA analysis. Animals were then fixed in 96% ethanol or 10% formalin, and transferred to 70% ethanol for long-term storage. Additional tissue samples (toe clips or tail tips) were taken from select animals that were not collected as specimens.

Barcode sequences of a mitochondrial 16S rRNA or ND2 gene fragment were produced from a subset of the collected animals as a preliminary baseline for taxonomic verification of species identity; further sequencing is pending. Sequencing work was done by the team in the Vences Lab at Technische Universität Braunschweig. Total genomic DNA was extracted using a standard salt extraction protocol (Bruford et al. 1992). Amplification of the 16S fragment was performed using standard primers for Madagascan herpetofauna (Vieites et al. 2009), except for chameleons, which were sequenced for the ND2 mitochondrial gene in line with recent and on-going work on their taxonomy (Gehring et al. 2012, Proetzl et al. submitted). Sequences were manually cleaned, and subsequently blasted against the NCBI database or placed in the context of species trees based on barcode data to assess species identity. Candidate species assessment is based on the thresholds used by Vieites et al. (2009).



Mark D. Scherz examines a specimen. (Photo by D. Parker)

4.8 Access and Benefits Sharing

Individual specimens of common species were donated to the University of Antsiranana, to form the foundation of a new herpetological museum collection in Antsiranana. Half of the remaining animals were deposited in the zoological collection of the Université d'Antananarivo Département de Biologie Animal (UADBA). The other half was deposited in the Zoologische Staatssammlung München (ZSM). All tissue samples were exported to the ZSM for DNA analysis and long-term deposition. Malagasy team members will be included in all peer-reviewed articles for which data from this expedition forms the foundation.

Our research was conducted under permit N°. 224/15/MEEMF/SG/DGF/DAPT/SCBT. Specimens were exported under permits N°s. 030N-EA01/MG16 and 029C-EA01/MG16.



A specimen of *Gephyromantis* sp. nov. 2 (aff. *tandroka*) (Photo by M.D. Scherz)

5. Results

A total of 46 species were recorded over the 22 days of survey work on the Ampotisdya Mountains. Transect surveys recorded 42 species over 702 separate observations. In addition, four further species were collected opportunistically. Four of the recorded species appear to be new to science and eight are currently undescribed.

5.1 Randomization analysis

Of the 702 recorded observations, 618 could be identified to subgenus level—or genus level where subgenera do not exist; data are reported in Table 2 and plotted in Figure 5). *Calumma* was by far the most frequently encountered group, with 295 specimens of five species. Rivers and streams were intersected at 18 positions over 15 different transects. These varied from 0.5 to 4 m wide. Mean distance from the forest edge was 47 m (sd=26.3), though there were no rivers between 70 and 90 m.

Table 2. Species distribution statistics at subgenus level.

Genus (Subgenus)	Samples (n)	Mean	SD	P-value
<i>Aglyptodactylus</i>	9	44.83	38.01	0.28
<i>Mantidactylus (Duboimantis)</i>	27	47.43	26.70	0.32
<i>Boophis</i>	31	34.06	24.44	0.00*
<i>Brookesia</i>	26	51.98	25.06	0.62
<i>Mantidactylus (Brygoomantis)</i>	10	29.23	23.13	0.01*
<i>Calumma</i>	295	51.61	26.79	0.84
<i>Mantidactylus (Chonomantis)</i>	17	38.26	12.56	0.06*
<i>Furcifer</i>	16	26.47	14.13	0.10*
<i>Gephyromantis (Laurentomantis)</i>	1	89.90	-	-
<i>Lygodactylus</i>	17	34.28	26.69	0.02*
<i>Madascincus</i>	2	12.25	0.35	0.03*
<i>Mantidactylus (Mantidactylus)</i>	7	54.01	28.23	0.62
<i>Mantidactylus (Ochthomantis)</i>	23	44.88	23.18	0.19
<i>Guibemantis (Pandanusicola)</i>	18	17.42	12.97	0.00*
<i>Phelsuma</i>	26	40.23	30.72	0.06*
<i>Platypelis</i>	12	48.89	25.23	0.44
<i>Plethodontohyla</i>	2	63.40	11.60	0.71
<i>Spinomantis</i>	17	61.15	28.94	0.95*
<i>Stumpffia</i>	3	75.33	29.02	0.92*
<i>Trachylepis</i>	15	46.49	24.29	0.37
<i>Uroplatus</i>	11	49.63	31.43	0.50
<i>Zonosaurus</i>	33	56.76	36.10	0.89
<i>Unclassified</i>	70	45.44	26.70	-

Significant P-values are denoted by *

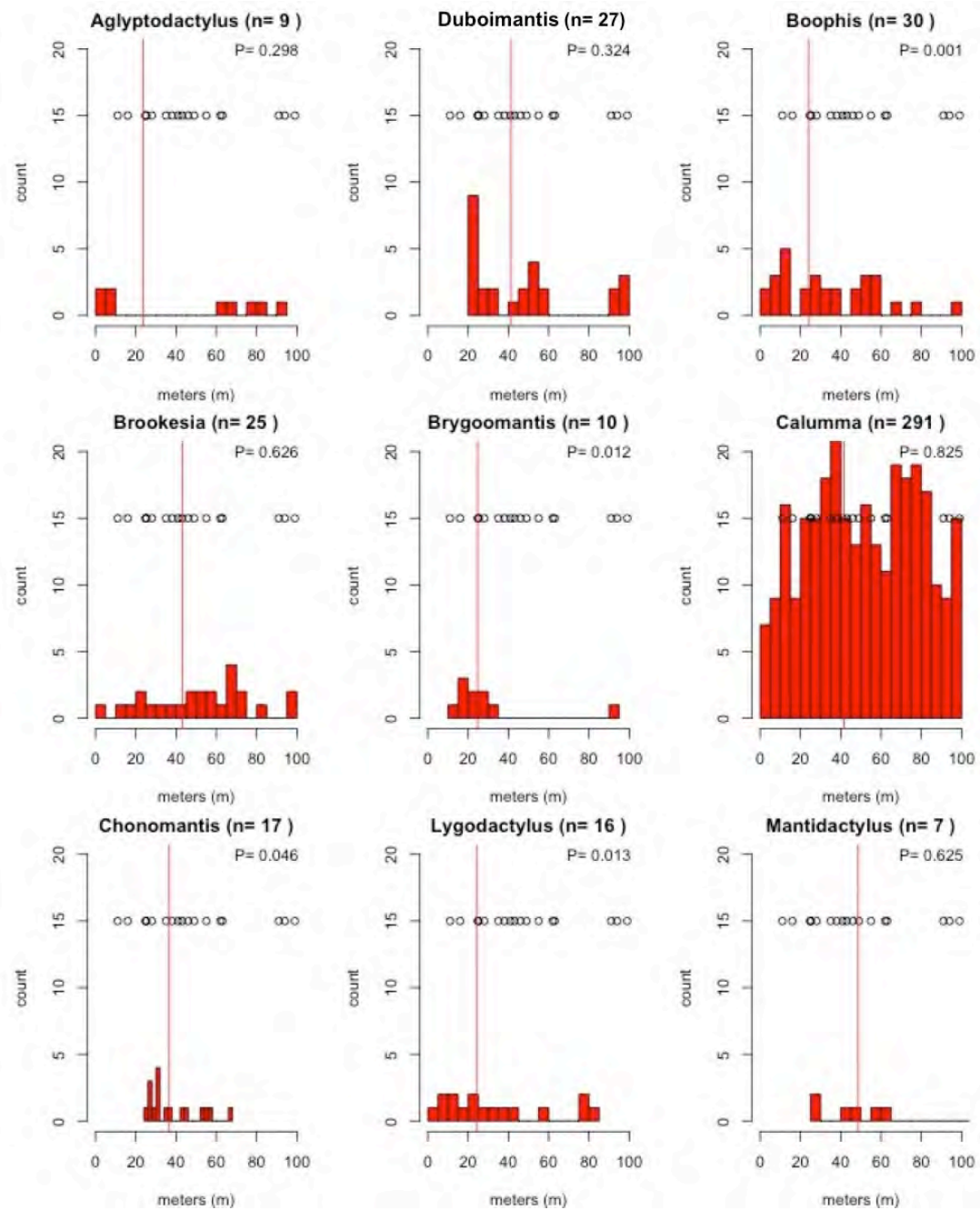


Figure 5. Distribution of subgenus level observations. Histograms denote raw observational data. Vertical line gives the geometric mean. P value compares true mean with a randomized distribution. Open circles mark the positions of rivers and streams.

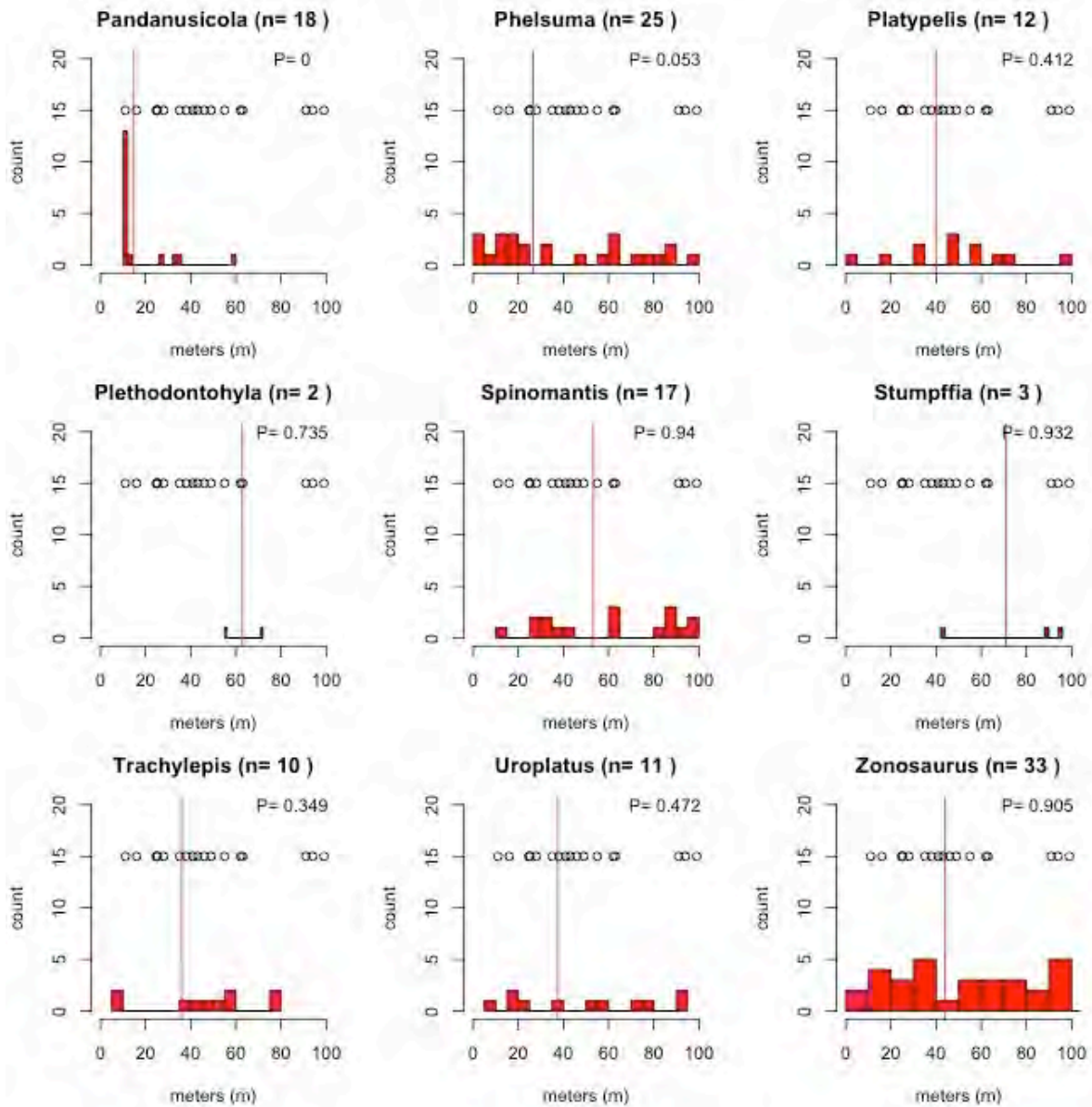


Figure 5. cont.

5.2 Rapid Habitat Assessment

The number of *Pandanus* heads was significantly different between edge quadrats (-20 and 5 m) and quadrats at 100 m into the forest. This suggests that in our research area, forest edges could have a deleterious impact on the survival of *Pandanus* plants, and *Pandanus*-specialist herpetofauna, beyond 50 m into the forest. Similarly, understory was found to vary significantly with distance ($X^2 p < 0.001$, $X^2_{simP} p < 0.001$, Fisher's Exact Test $p < 0.001$). However, tests on just the within-forest data at 5 m, 50 m and 100 m resulted in no significance ($X^2 p = 0.6418$, $X^2_{simP} p = 0.7456$, Fisher's $p = 0.7354$). Most transects were classified as having a medium or sparse understory at a distance of 5 m and greater into the forest. Primary rainforest tends towards a sparser understory (Malcolm 1994; Murcia 1995) and concordantly these results illustrate the abruptness of the primary forest edge on which we were working. The volume of deadfall did not vary significantly in any category, but was generally considered to be abundant.

Canopy cover increased along transects, with statistical significance between -20 m and 50 m but non-significance between 50 m and 100 m. The number of large trees did not vary significantly at different distances within the forest and no significant difference was found between tree circumference at breast height and distance from the forest edge. This suggests that trees at a distance into the forest of 50 m or greater have more canopy foliage, either due to the species composition or physiological factors.

Whilst cut stumps were mostly absent from the research area, burning appeared to be the dominant method for forest clearance. The categorical data tests indicated that evidence of burning varied significantly with distance ($X^2 p < 0.001$, $X^2_{simP} p < 0.001$). Tests on just the within-forest data at 5 m, 50 m, and 100 m also gave significant results ($X^2 p < 0.01$, $X^2_{simP} p < 0.001$, Fisher's $p < 0.001$). Between 50 m and 100 m however the result was not significant. Burning evidence was generally absent at a distance greater than 50 m from the edge and was only present at 5 m in to the forest on six transects. Burning evidence was recorded at >50 m on two transects. These transects grew closer to the fragment edges at various distances along the transect, despite beginning perpendicular to the forest edge.

The abundance of bracken was significantly higher at -20 m than at any other distance. No significant difference was observed between the other distances indicating that bracken can only penetrate a few meters into the forest habitat. No variation in ground fern abundance was observed with distance from the forest edge, however the species composition of ferns was observed to differ with large forest ferns only persisting in the least disturbed primary forest habitats.

Leaf litter depth increased significantly between -20 and 50 m, but no significant difference was observed between 50 and 100 m.

In summary, no significant variation in the habitat measurements were observed between 50 and 100 m into the forest. This suggests that edge effects have less influence on forest habitat beyond a distance of 50 m. There is the possibility that edge effects on forest habitat penetrate to a greater depth than 100 m but were missed in this study, but evidence from other studies would suggest this is not the case, and in most studies transects of 50 m have been used (DeMaynadier & Hunter 1998; Lehtinen et al. 2003; Jenkins et al. 2003).

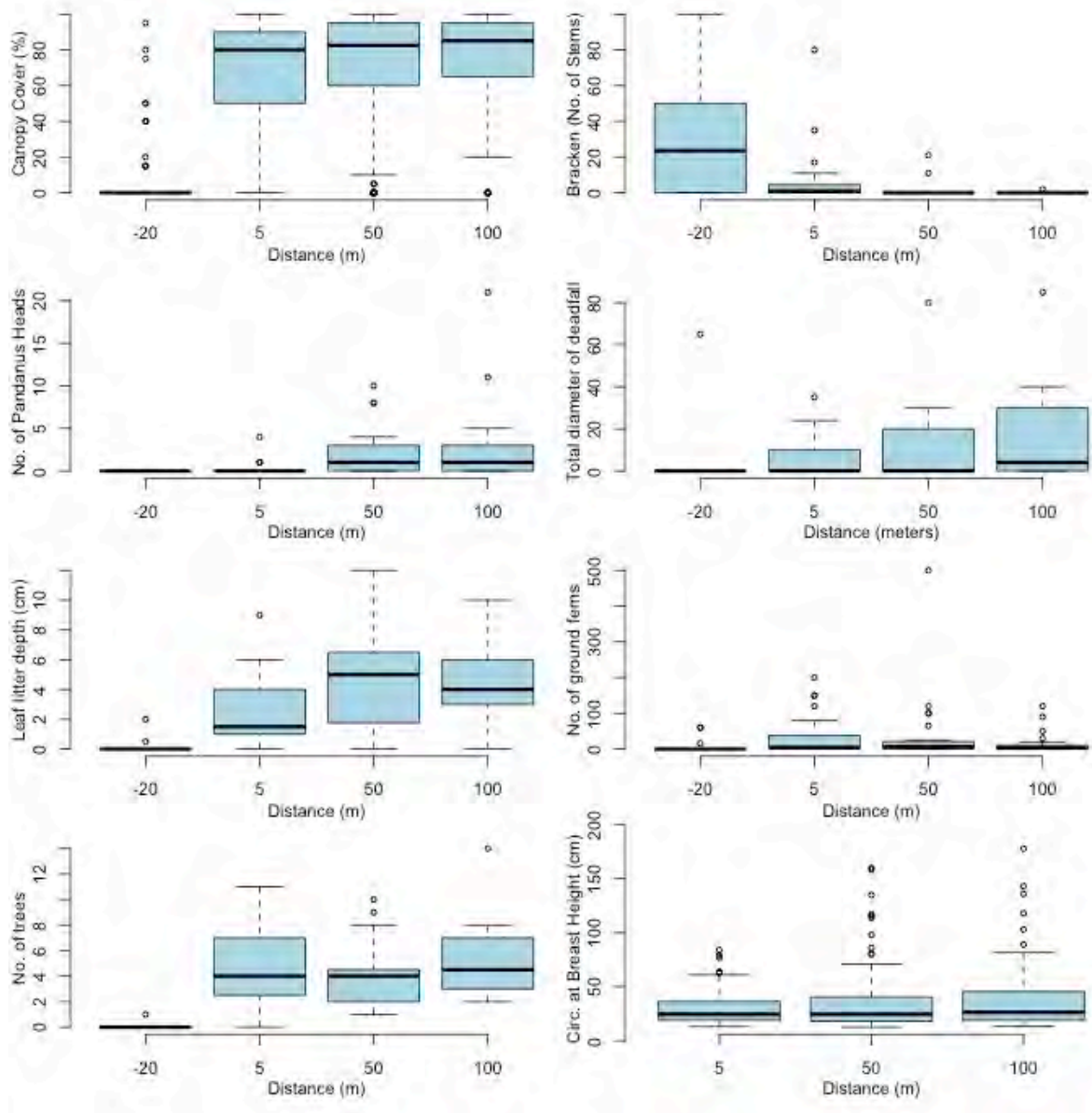


Figure 6. Box plots of rapid habitat assessment data at -20, 5, 50 and 100 m. There were no trees beyond the forest edge, thus the -20 m distance class is excluded for circumference at breast height.

5.3 Microclimate Assessment

Temperature traces at 10 m intervals over a 36 hour period on transect 19 are illustrated in figure 7 below. Temperatures in the evening and overnight from 1900 to 0600 h (hours 7-18) remained relatively constant throughout the transect at around 18 °C, with lows observed around dawn. Throughout the morning and at midday, edge temperatures until approximately 20–30 m can be seen to rise faster and to a higher maximum. Sunrise on this day was approximately 0515 h local time, and sunset approximately 1815 h, though true times probably differed due to the topography of the region. We hypothesise that brief dips in temperature are associated with rainfall events. Descriptive statistics are given in Table 3.

The lowest temperature recorded on transect 19 was 16.5 °C, and consistent across several distances. This rose to a high of 31.5 °C at 0 m shortly after midday. Both mean and average deviation were highest on the edge and broadly declined into the forest. Results appear to become broadly consistent after 30m. Boxplots (Figure 8) summarise data for each distance across all transects showing higher variability and mean temperature towards the forest edge. The results of this study will be analysed in greater detail for publication alongside our higher resolution sampling data on species distribution.

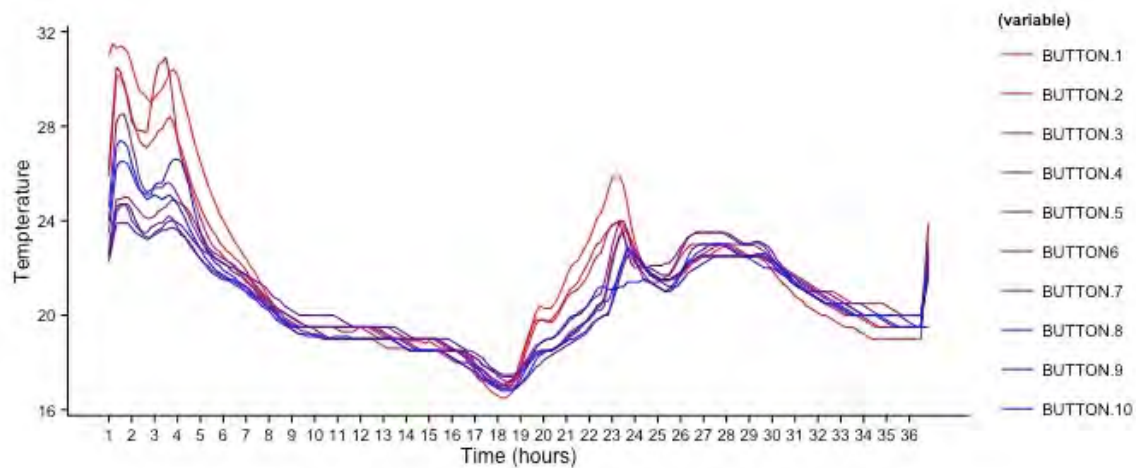


Figure 7. Transect 19 diurnal temperature variation (36 hour period, commencing 1200 noon)

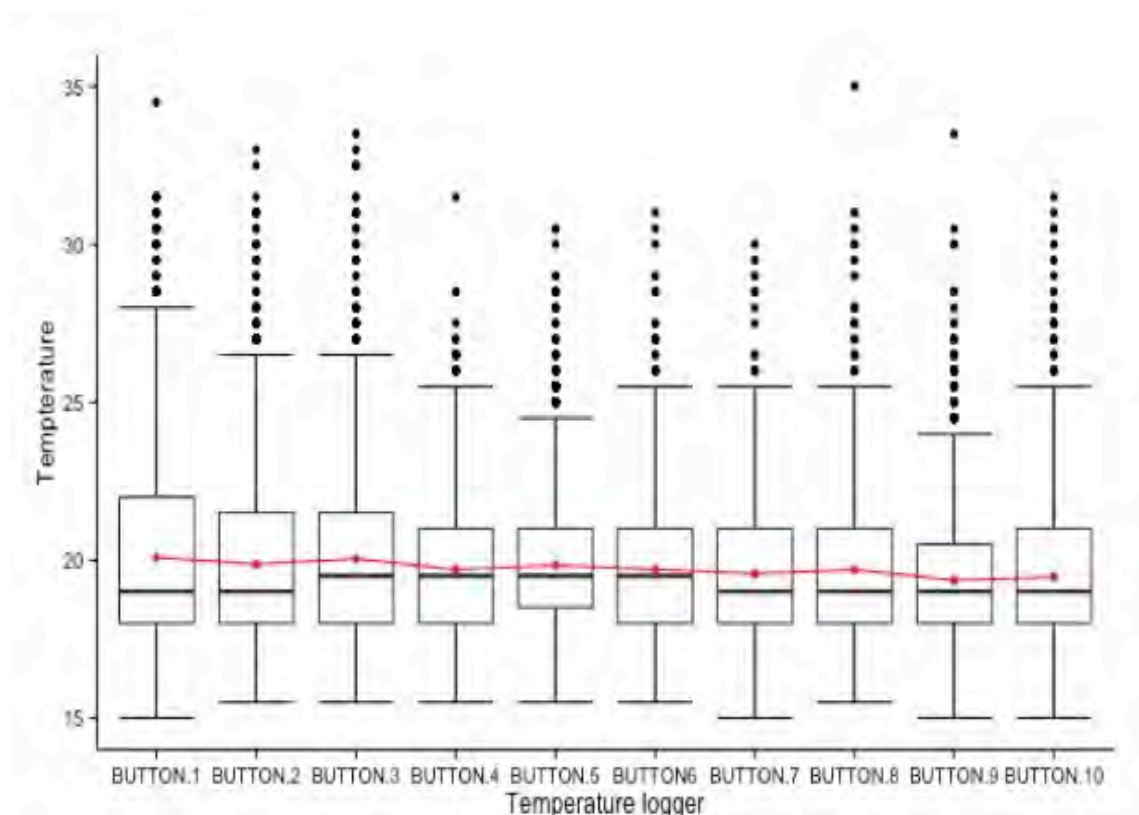


Figure 8. Boxplots of 36 hour temperature variation from 0–90m across 10 transects.

Table 3. Descriptive statistics for Transect 19 temperature data (36 hour period)

	0 m	10 m	20 m	30 m	40 m	50 m	60 m	70 m	80 m	90 m
Maximum	31.5	31.0	33.5	25.5	30.0	26.0	25.0	24.0	27.5	28.0
Minimum	16.5	16.5	17.0	17.0	17.0	17.0	17.0	17.5	16.5	16.5
Mean	21.7	21.3	21.5	20.8	21.2	20.8	20.6	20.7	20.7	20.7
Ave. Dev.	2.7	2.3	2.2	1.8	2.0	1.7	1.7	1.5	1.9	1.8

5.4 Drone imagery and orthomosaic maps

The expedition succeeded in undertaking a small number of flights over an area approximately 600 m by 200 m. Wind conditions were unexpectedly challenging, possibly due to the topography of the study site. Figure 9 provides a preliminary look at our proposed proof of concept analysis. Further work will attempt to assess changes in canopy structure using these models.

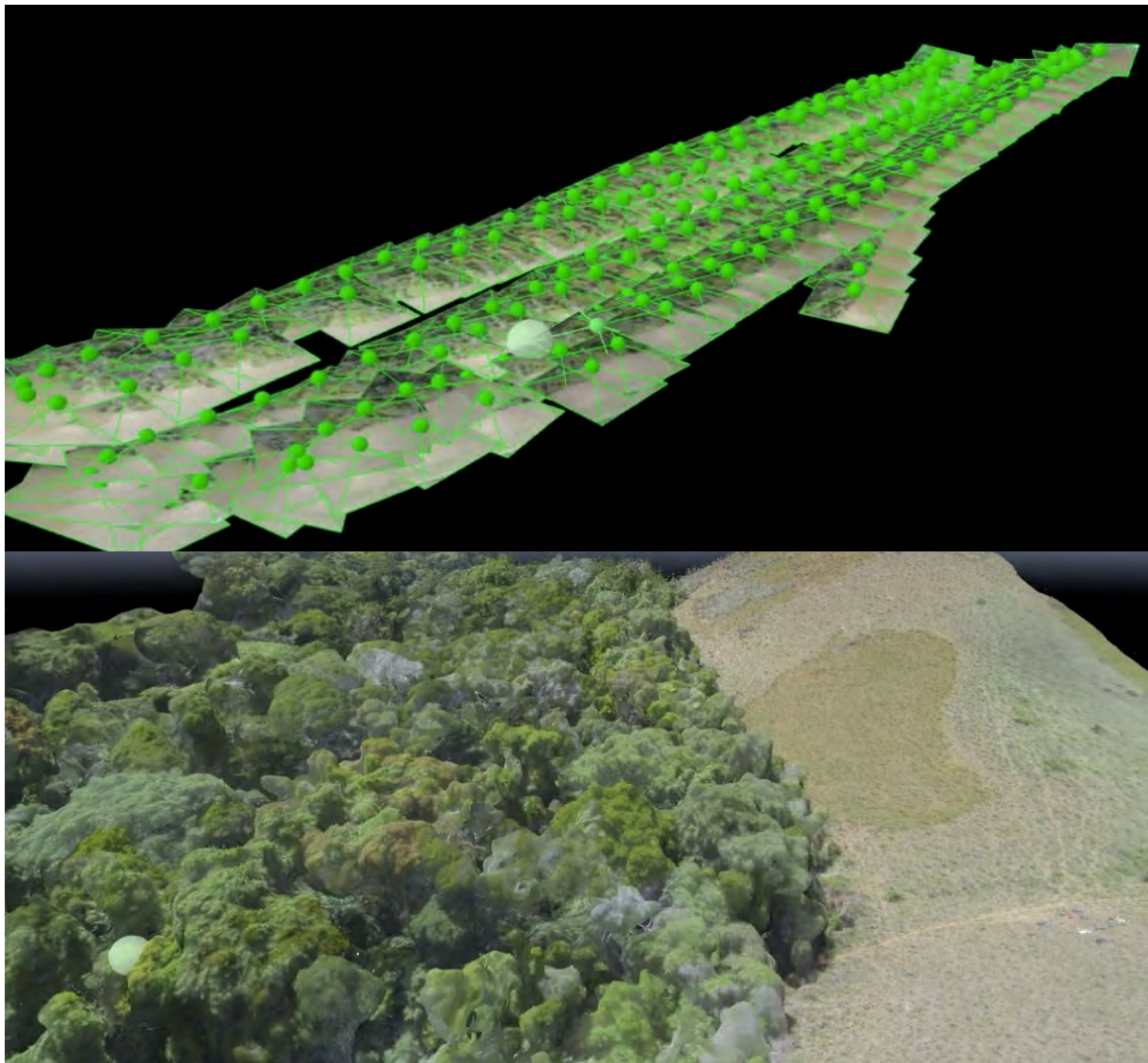


Figure 9. Building a model of canopy structure from ~600 aerial images.

5.5 Herpetofaunal Inventory and collections

A total of 186 specimens of 46 species were collected over the 22 days of survey work on the Ampotsidy mountains. Species diversity was 56.5% (n = 26) for frogs, and 43.5% (n = 20) for reptiles (see Table 4). Of the frogs, 3.8% (n = 1) were reed frogs (family Hyperoliidae), 3.8% (n = 1) were grass frogs (family Ptychadenidae), 15.4% (n = 4) were narrow-mouthed frogs (family Microhylidae), and 76.9% (n = 20) were mantellids (family Mantellidae). Of the reptiles, 30% (n = 6) were geckos (family Gekkonidae), 15% (n = 3) skinks (family Scincidae), 45% (n = 9) chameleons (family Chameleonidae), 5% (n = 1) plated lizards (family Gerrhosauridae), and 5% (n = 1) lamprophiid snakes (family Lamprophiidae).

Several species were certainly missed by our surveys. A single individual of *Boophis tephraeomystax* was caught but escaped, and could not be recollected. Local people reported that boas (*Acrantophis* or *Sanzinia*) were found in the area, but none were seen during our surveys. A second species of plated lizard (*Zonosaurus laticaudatus*) may have been present, but was not captured and cannot be verified.

Additionally, several species of frog were heard but not caught, and it is therefore clear that our inventory of the herpetofauna of the Ampotsidy Mountains is still incomplete. Furthermore the discovery of several taxa on the final few days of surveying indicates that more research on these forests will reveal additional taxa. In particular, we suspect that numerous fossorial taxa were missed by our surveys.

Of the taxa captured, at least four are new species to science (see Table 4). Three of these qualify as confirmed candidate species (following the criteria of Vieites et al. 2009), due to call differences with described species and other candidates, while the fourth is an unconfirmed candidate species pending further investigation. Eight species encountered are known undescribed species (see Table 5). DNA sequence data have not yet been produced from most reptiles, and several amphibians also remain to be sequenced. However, we may preliminarily conclude that 17.4% of all species encountered are undescribed taxa, and 8.7% previously unknown to science. We collected the first adult specimens of *Boophis* sp. Ca49 and *B.* sp. CaNEW, which had previously been known only from tadpoles.

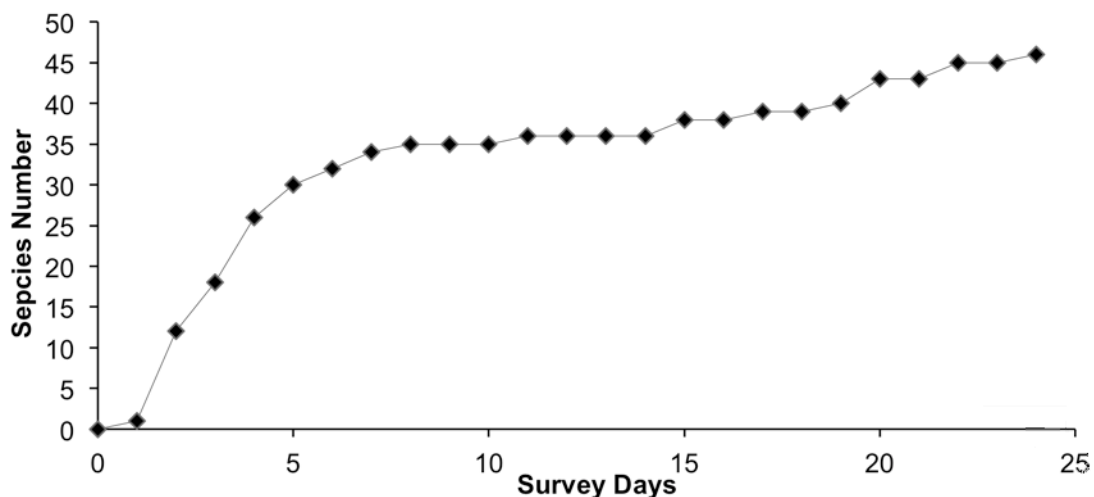


Figure 10. Accumulation curve of specimens collected during our surveys. The curve begins to plateau after roughly five days but continues to rise until the 24th (last) day of sampling.

In addition to confirmed and potentially new species, our research provided new locality records for all of the included species (because the herpetofauna of the forests of Ampotsidy have never been studied previously), marking particularly significant range extensions in *Boophis goudoti* and *B. guibei*. The range of *Plethodontohyla guentheri* is also extended west here, being previously known only from eastern Madagascar (Glaw & Vences 2007; M. D. Scherz unpubl. data). These are ecologically important range extensions with significant conservation implications, and will be formally published at a later date.

Table 4. Species recovered by our surveys in Ampotsidy. Ecological codes: T = terrestrial, A = arboreal, F = fossorial. Habitat codes: F = forest, G = grassland, M = grassy marsh. Microhabitat codes: L = leaf litter, R = riparian, S = slow water, F = forest away from water, E = edge, D = disturbed or secondary vegetation, M = grassy marsh, G = grassland. Red List Status: NA = not assessed, DD = Data Deficient, LC = Least Concern, NT = Near Threatened, VU = Vulnerable, EN = Endangered, CR = Critically Endangered (accessed 4 April 2016). Records from other areas refer to these papers: [1] Andreone et al. 2009; [2] Glaw & Vences, 2007; [3] Rabearivony et al. 2010; [4] Vieites et al. 2009; [5] Rakotomalala 2002; [6] Vences et al. in review; [7] Raxworthy & Nussbaum 1996; [8] Vences et al. 2002; [9] Rabearivony et al. 2015; [10] Ratsoavina et al. 2015; [11] Florio et al. 2012. Range extensions are only extensions of ≥ 100 km.

FROGS	Ecology	Habitat	Micro-habitat	IUCN Red List Status	Previous record from Tsaratanana	Previous record from Manongarivo	Previous record from Bemanevika	Range extension?
Mantellidae								
<i>Aglyptodactylus madagascariensis</i>	T	F	L	LC		[1]*	[3]	
<i>Boophis goudoti</i>	A	F	R	LC				+
<i>Boophis guibei</i>	A	F	F	LC				+
<i>Boophis madagascariensis</i>	A	F	S	LC			[3]	
<i>Boophis</i> sp. nov. 1 (aff. <i>marojezensis</i>)	A	F	R					
<i>Boophis</i> sp. Ca49 (aff. <i>sambirano</i>)	A	F	R					
<i>Boophis septentrionalis</i>	A	F	R	DD	[1,2,9]	[1]		
<i>Boophis</i> sp. CaNEW (aff. <i>entingae</i>)	A	F	D,R		[unpubl]			
<i>Gephyromantis (Laurentomantis) horridus</i>	A,T	F	F	EN	[1,8]			
<i>Gephyromantis</i> sp. nov. 1 (aff. <i>asper</i>)	A,T	F	S					
<i>Gephyromantis</i> sp. nov. 2 (aff. <i>tandroka</i>)	A,T	F	F					
<i>Gephyromantis tahotra</i>	A,T	F	F	NA			[6]	
<i>Guibemantis (Pandamusicola) liber</i>	A	F	S,R	LC			[3]	
<i>Mantidactylus (Ochthomantis) ambreensis</i>	T	F	R	LC	[1,2,9]	[1,2,9]		
<i>Mantidactylus (Ochthomantis) femoralis</i>	T	F	R	LC	[1]	[1,5]	[3]	
<i>Mantidactylus (Mantidactylus) guttulatus</i>	T	F	R	LC	[2,9]	[5]	[3]	
<i>Mantidactylus (Chonomantis) sp. Ca3</i>	T	F	R					
<i>Mantidactylus (Ochthomantis) sp. Ca45</i>	T	F	R			[4]		
<i>Mantidactylus (Brygoomantis) cf. biporus</i>	T	F	S	LC	[1,9]	[1]	[3]	
<i>Spinomantis peraccae</i>	A	F	R	LC	[1:'sp. aff',2,9]	[1:'sp. aff',5]	[3]	

	Ecology	Habitat	Micro-habitat	IUCN Red List Status	Previous record from Tsaratanana	Previous record from Manongarivo	Previous record from Bemanevika	Range extension?
FROGS								
Hyperoliidae								
<i>Heterixalus betsileo</i>	A	M	M	LC				+
Microhylidae								
<i>Platypelis grandis</i>	A	F	F	LC		[5,9]		
<i>Platypelis</i> sp. Ca10	A	F	F			[1]†		
<i>Plethodontohyla guentheri</i>	T	F	F	DD				+
<i>Stumpffia</i> sp. (cf. <i>tetradactyla</i>)	T	F	L					
Ptychadenidae								
<i>Ptychadena mascareniensis</i>	T	M	M	LC	[1,2]		[3]	
REPTILES								
Lamprophiidae								
<i>Leioheterodon madagascariensis</i>	T	G	E,G	LC				
Gekkonidae								
<i>Lygodactylus madagascariensis</i>	A	F	F	VU	[1]	[1,5]		
<i>Lygodactylus miops</i>	A	F	F	LC				
<i>Lygodactylus</i> sp.	A	F	F					
<i>Phelsuma lineata</i>	A	F	F,E	LC	[2,7,9]	[5]		
<i>Uroplatus sikorae</i>	A	F	F	LC	[1,2,9]	[1,2,9]		
<i>Uroplatus</i> sp. Ca1	A	F	F		[10]		[10]	
Scincidae								
<i>Madascincus melanopleura</i>	F	F	L	LC	[1,2]	[5]		
<i>Madascincus mouroundavae</i>	F	F	L	LC	[1,2,9]	[5]		
<i>Trachylepis gravenhorstii</i>	T	G	G,E	LC		[2]		
Chameleonidae								
<i>Calumma brevicorne</i> ◊	A	F	F	LC				
<i>Calumma crypticum</i> ◊	A	F	F	LC				
<i>Calumma guillaumeti</i>	A	F	F	LC	[1,2,9]	[1]		
<i>Calumma</i> sp. nov. aff. <i>nasutum</i>	A	F	F					
<i>Calumma</i> sp. nov. aff. <i>guibei</i>	A	F	F		[2]			
<i>Furcifer viridis</i>	A	G	G	LC	[12]		[12]	
<i>Furcifer willsii</i>	A	F	F	LC	[2,9]			
<i>Brookesia</i> sp. 1 (cf. <i>thieli</i>)	T	F	L	EN	[1]			
<i>Brookesia</i> sp. 2 (cf. <i>betschi</i>)	T	F	L	EN				
Gerrhosauridae								
<i>Zonosaurus madagascariensis</i>	T	F	F	LC		[1,2,5,9]		

* Andreone et al. 2009 report *A. securifer*, which could be confused with this species.

† Andreone et al. 2009 report four candidate species of *Platypelis*, which may include this species, but examination of some of their specimens housed in the Zoologische Staatssammlung München suggests that they do not.

◊ The identity of these species is in need of further clarification; see below. They are undoubtedly conspecific with species found in nearby areas (e.g. refs. 1–3), but are more diverse than anticipated.

One of the target species for our research was the chameleon *Calumma hafahafa*, from the *C. brevicorne* species complex. Regrettably, we were not able to find any individuals of this species, although numerous individuals from the *C. brevicorne* complex were found. The genetic data on these specimens remains to be assessed in detail, but indicates that at least some individuals may belong to *C. amber*, *C. peltierorum*, or *C. hilleniusi*; more extensive sequencing of collected tissue samples will be required to resolve the identity of all of the collected species. Although our surveys were not exhaustive, chameleons are among the easiest taxa to locate on surveys (due to their reflective skin at night), and we therefore conclude that it is unlikely that *C. hafahafa* is present in these fragmented forests. This species may be strongly associated with particular microhabitats that were either not

surveyed or are not present on the Ampotisy Mountains. Further research into the ecology of this species is needed before conclusions can be drawn.

Table 5. Candidate species discovered at Ampotisy. Bolded species are new discoveries. CCS = confirmed candidate species, UCS = unconfirmed candidate species, KUS = known undescribed species. Note that the status of most reptiles has not yet been ascertained.

Species	Candidate Status	Rationale
<i>Boophis</i> sp. nov. 1	CCS	3% 16S divergence from <i>Boophis</i> sp. Ca26 (from the <i>B. marojezensis</i> complex within the <i>B. majori</i> group), small call differences to described species.
<i>Gephyromantis</i> sp. nov. 1	CCS	4% 16S divergence from <i>G. asper</i> , highly distinct call
<i>Gephyromantis</i> sp. nov. 2	CCS	7% 16S divergence from <i>G. tandroka</i> and small call differences to all spp.
<i>Stumpffia</i> sp. nov. 1	UCS	7% 16S divergence from <i>S. pygmaea</i> . More sequence data are needed to confirm the status of this species.
<i>Calumma</i> sp. nov. 1	KUS; CCS	Highly divergent from all described <i>Calumma</i> species in the ND2 mitochondrial fragment; clade I sensu Gehring et al. (2012).
<i>Calumma</i> sp. nov. aff. <i>guibei</i>	KUS; CCS	Under description by D. Prötzel et al.
<i>Uroplatus</i> sp. Ca1	KUS; CCS	Under description by P.-S. Gehring et al.
<i>Mantidactylus</i> sp. Ca3	KUS; CCS	Description status not known.
<i>Boophis</i> sp. CaNEW	KUS; CCS	3–4% 16S divergence from <i>Boophis</i> sp. Ca42. Previously known; identical to ZCMV 13143 tadpole
<i>Boophis</i> sp. Ca49	KUS; CCS	Not under description. First adult specimens.
<i>Mantidactylus</i> sp. Ca45	KUS; CCS	Description status not known.
<i>Platypelis</i> sp. Ca10	KUS; CCS	Not under description.

6. Discussion

The herpetofaunal diversity of the forests of Ampotisy is remarkable. Almost 50 species are found within partly fragmented and disturbed primary and secondary forest. Over 17% of all taxa encountered are apparently new to science. Although Madagascar remains a hotspot for species discovery, the reptiles and amphibians have been subject to extremely intense study over the last 20 years, and surveys with such a high percentage of scientifically unknown taxa are increasingly rare (but similar values have recently been achieved by C. Hutter and S. Lambert in eastern Madagascar, C. Hutter pers. comm.). It is clear that the forests of the Sofia region of northern Madagascar need more extensive herpetological work.

6.1 Microclimate and Habitat Surveys

The data clearly show higher mean temperatures, higher variability and higher rates of temperature change in data loggers positioned closer to the edge. This conforms to previously published data

showing a detectable edge influence up to 30–40 m into the forest. Unsurprisingly, habitat surveys show the clearest differences between -20 m and 5 m quadrats. The lack of any kind of plug vegetation to seal the forest edges was surprising. While some indicators such as canopy cover and number of trees appear to remain relatively consistent once inside the forest, others such as leaf litter depth and deadfall continue to increase at 50 m or more. It appears that abiotic environmental factors transition abruptly and have a narrow edge region, while biotic factors change more gradually, and their edge effect extends over 50 m. This suggests a high complexity to the edge factors affecting native herpetofauna, whose distribution is strongly influenced by both biotic and abiotic factors (see below).

6.2 Species Distributions and Edge Effects

Several species groups displayed strong edge-biased distributions. *Boophis*, the *Mantidactylus* subgenera *Brygoomantis* and *Chonomantis*, and *Guibemantis* (*Pandanusicola*) amphibian groups all favoured edges. This result is surprising; of these groups, only the ecology of a few species of *Boophis* can be thought of as ‘edge-tolerant’; *Chonomantis* males are almost always found in close proximity to flowing water (and only two females were encountered, albeit both further away from water, but also both at least 20 m from the forest edge), and *Brygoomantis* are some of Madagascar’s most aquatic frogs, generally sitting in or directly beside still or slow-flowing water. The only species of *Pandanusicola* found, *Guibemantis* (*Pandanusicola*) *liber*, is generally dependent on *Pandanus* for its survival (though it is not as strongly so as other members of the subgenus, and we observed females laying eggs on non-*Pandanus* plants; Glaw & Vences 2007). As we have shown, there was lower *Pandanus* abundance near the edges of the studied forests. Inspection of our data reveals however a single exceptional transect, number 15, where this species was encountered in large numbers near to the edge of the forest, coinciding also with a near-edge *Pandanus* stand. Thus, the abundance of all of these groups except *Boophis* near the edge is likely confounded by the position of rivers, which were generally nearer to the zero point of the transect than the 100 m point (see Figure 5).

Surprisingly, although *Spinomantis peraccae* (the only encountered member of the genus) is just as strongly associated with *Pandanus* as *Guibemantis* (*Pandanusicola*) *liber*, it was found to be relatively strongly associated with the interior of the forest. Unlike *G. liber*, the tadpoles of which are relatively weak swimmers and typically fall from leaves into slow-flowing or almost still water, the tadpoles of *S. peraccae* are adapted to fast-flowing water (Ndriantsoa et al. 2012). The speed of the streams may therefore explain why *S. peraccae* was found near the streams deep in the forest and *G. liber* was not; we did not measure stream flow rate. As for their abundance, unlike *G. liber*, *S. peraccae* typically occurs at fairly low density, with a few males per 10 m² (females were not encountered)—*G. liber* can occur at densities of several dozen individuals of both sexes within the same area. Thus, the number of *S. peraccae* individuals encountered in total was fairly low (limited by the search distance either side of the transect line) relative to *G. liber*, even though many individuals could often be heard (the call of *S. peraccae* is highly distinct and easily recognizable over great distance).

Two groups in particular, *Mantidactylus* subgenera *Ochthomantis* and *Mantidactylus* are 100% explained by the position of rivers. *Ochthomantis* are almost never found more than 10 m from a river, and subgenus *Mantidactylus* in fact lives almost entirely within rivers and streams. Based on observations by M.D. Scherz from other parts of the Sofia region, it is likely that these taxa (especially subgenus *Mantidactylus*) are able to persist in streams as long as the riparian habitat

(bushes and trees) along the streams is at least one to two metres wide—presumably because it functions as a humidity buffer and provides shade. A similar pattern to *Ochthomantis* was observed in *Duboimantis* species, but as these are less dependent on streams (and only breed near or in slow-flowing water), this pattern cannot be so readily explained.

Among the reptiles, *Furcifer*, *Madascincus* (small sample size), and *Phelsuma* also favoured edges. Of the two *Furcifer* species encountered, one, *F. viridis*, lives almost exclusively outside forest, and the other, *F. willsi* is a deep-forest specialist (Glaw & Vences 2007)—the latter represents a negligible fraction of the encountered *Furcifer* specimens, and we may conclude that *F. viridis* is indeed an open savannah specialist, with two fluke specimens having been encountered within the forest. Opportunistic surveys whilst traversing the open areas between forest at night frequently encountered in excess of 50 individuals of this species within 30 minutes, and it is probably the most abundant reptile in the entire region. Only one *Phelsuma* species, *P. lineata*, was encountered. This species is typically found in anthropogenically altered habitats, and is highly tolerant of disturbance (although it still requires relatively high humidity). Thus, its presence outside of the forest is also no surprise. However, our *Madascincus* result is surprising. This genus consists of fossorial and semi-fossorial skinks. We posit that our finding of it near the edge of the forest reflects the relatively shallow layer of leaf litter, making it easier to detect toward the edge than at the centre. This is supported by our having encountered three specimens of *Madascincus* whilst clearing a space for basecamp approximately 50 m from the forest edge and in excess of ten individuals in the pitfall traps, at ~100 m within the forest.

In summary, our data indicate strong taxon-specific impact of edge effects on distribution, which are generally explainable by knowledge of the ecology of the groups, with a few exceptions. This highlights the importance of ecological knowledge in the formulation of conservation strategies at the ecosystem- and species-specific level. However, as we have shown, this fundamental knowledge for effective management can be gained by rapid rainy-season surveys, and this need not be a barrier standing in the way of conservation action.

7. Expected Outcomes and Prospects

Our research outcomes will include several peer-reviewed scientific publications, as well as a number of presentations and popular science articles. Peer-reviewed publications are expected to include at least one ecological paper based on a thorough analysis of our data using DISTANCE methods, and several taxonomic contributions (or part of these). Several specimens we collected have been included in taxonomic works that were already underway, including a new chameleon species (Prötzel et al. submitted) and probably a new gecko species (Gehring et al. in prep). A description of one of the frog species that we discovered will be published by the team in 2017 (Scherz et al. in prep). A subset of the reported data is being used as part of two Masters Theses by the Malagasy students, and species-specific data will be used to inform IUCN Red Listing of amphibian species.

7.1 Further Opportunities

Whilst the wet season was selected as the most appropriate time for fieldwork, it is possible that dry season conditions may pose as a limiting factor for some species. Thus, future work might include a

reciprocal study in June/July. Furthermore, our species accumulation curve (Figure 10) indicates that sampling is not complete, and several additional species probably remain to be discovered. Particularly conspicuous by their absence were snakes, but also several fossorial and semi-fossorial lizards that were expected were not recovered. In this case, we interpret this not as a strong aversion to edge regions, but rather to low frequency, strong environmental dependence, and general difficulties in surveying these taxa.

Several of the amphibians in the region have loud calls that can be heard even outside the forest at significant distances. It may therefore be possible to implement remote sensing in the form of temporary recorders in order to assess the distribution of some species, most notably members of the genus *Gephyromantis*, and *Boophis madagascariensis*, the latter of which can be heard at a distance of 150 metres outside the forest with low winds. This method may enable partial surveys of forest fragments that are difficult to gain physical access to due to their location and topography.

It is clear from our preliminary analysis of the data that several taxa are tied closely to environmental conditions, and that this can be a strongly confounding factor in our analyses; the ecology of the species in most cases explains their distribution more convincingly than putative edge effects. In order to confirm edge effects with greater confidence, stronger controls are required for environmental variation along transects, most notably for the position of rivers, which have a strong effect on the distribution of most frogs. As reptiles are less strongly influenced by water features, these probably make better candidates for understanding edge effects, but greater sample sizes than those that we were able to produce (except possibly for the genus *Calumma*, which will certainly require finer analysis) will be needed to understand edge effects in these species.

Finally, we wish to highlight the benefit of long-term field studies focusing on edge effects in fragmented forests. Ideally, this would involve longitudinal surveys both pre-and post-disturbance, to identify how quickly species and community distribution responds to edge effects.



Boophis septentrionalis (Photo by M.D. Scherz)

Table 6. Breakdown of expenditure for Expedition Angano

ITEM	DETAILS	COST (GBP)
EQUIPMENT	Tents (10)	£140
	Malagasy camping equipment (4 persons)	£160
	Cooking equipment	£43
	Generator fuel + oil	£48
	Specimen preparation (ethanol, formalin etc)	£28
	Pitfall trapping	£31
	Camp equipment	£60
	Audio recording equipment	£350
	Drone FPV kit	£180
	Misc items (water purification, specimen bags, sheets etc.)	£73
	DATA STORAGE	2x2TB waterproof hard drives
	Laptop batteries	£40
	Deep cycle marine battery	£62
COMMUNICATIONS	SATELLITE phone airtime	£88
	Local sim cards and credit	£55
TRANSPORT	International flights	£3,840
	Excess baggage	£180
	MICET vehicle support	£680
	Vehicle fuel costs	£460
ACCOMMODATION:	Antananarivo	£92
	Accommodation en route	£70
	Driver Accommodation	£40
FOOD	In the field	£920
	En route + Tana	£110
MICET PERMITS	MICET permit facilitation	£1,110
	1x supervisor support fees	£300
	3x student support fees	£1,020
	Additional associated transport	£120
GUIDES/PORTERS	Full time guides	£250
	Camp cooks	£160
	Local porters	£260
	Ox carts (transport)	£36
	Donation to village for use of rooms/support	£50
	Village party	£74
	Local transport costs (resupplies etc)	£60
	MEDICAL	Insurance
	Medical supplies	£166

FILMING	Editing costs	£875
MISC	Visas	£180
	Crowdfunding costs	£110
	Website costs	£18
	Report printing	£50
	Exchange rate losses	£60
	Misc tips	£50
RESERVES	Research dissemination	£222
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	TOTAL	£13,766
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Uroplatus sp. Ca1 (Photo by M.D. Scherz)

9. References

- Andreone, F., Glaw, F., Mattioli, F., Jesu, R., Schimmenti, G., Randrianirina, J.E. & Vences, M. (2009) The peculiar herpetofauna of some Tsaratanana rainforests and its affinities with Manongarivo and other massifs and forests of northern Madagascar. *Italian Journal of Zoology*, 76, 92–110. 10.1080/11250000802088603
- Brown, J.L., Cameron, A., Yoder, A.D. & Vences, M. (2014) A necessarily complex model to explain the biogeography of the amphibians and reptiles of Madagascar. *Nature Communications*, 5, 5046. 10.1038/ncomms6046
- Brown, J.L., Sillero, N., Glaw, F., Parfait, B., Vieites, D.R. & Vences, M. (2016) Spatial Biodiversity Patterns of Madagascar's Amphibians and Reptiles. *PLoS ONE*, 11, e0144076. 10.1371/journal.pone.0144076
- Bruford, M.W., Hanotte, O., Brookefield, J.F.Y. & Burke, T. (1992) Single-locus and multilocus DNA fingerprint. *In: Hoelzel, A.R. (Ed.) Molecular Genetic Analysis of Populations: A Practical Approach*. IRL Press, Oxford, pp. 225–270.
- Cadenasso, M.L. & Pickett, S.T.A. (2001) Effect of edge structure on the flux of species into forest interiors. *Conservation Biology*, 15(1), 91–97.
- Camargo, J.L.C. & Kapos, V. (1995) Complex edge effects on soil moisture and microclimate in central Amazonian forest. *Journal of Tropical Ecology*, 11(2), 205–221.
- Chazdon, R.L. (2003) Tropical forest recovery: legacies of human impact and natural disturbances. *Perspectives in Plant Ecology, Evolution and Systematics*, 6(1-2), 51–71.
- Davies-Colley, R.J., Payne G.W.c & van Elswijk M. (2000) Microclimate gradients across a forest edge. *New Zealand Journal of Ecology*, 24(2), 111–121.
- DeMaynadier, P.G. & Hunter, Jr., M.L. (1998). Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. *Conservation Biology*, 12(2), 340–352.
- Didham, R.K. & Lawton, J.H. (1999) Edge Structure Determines the Magnitude of Changes in Microclimate and Vegetation Structure in Tropical Forest Fragments. *Biotropica*, 31(1), 17–30.
- Ewers, R.M. & Banks-Leite, C. (2013) Fragmentation Impairs the Microclimate Buffering Effect of Tropical Forests. *PLoS ONE*, 8(3), e0058093. 10.1371/journal.pone.0058093
- Fahrig, L. (2003). Effects of Habitat Fragmentation on Biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, 34(2003), 487–515.
- Gascon, C., Williamson, G.B. & da Fonseca, G.A.B. (2000) Receding Forest Edges and Vanishing Reserves. *Science*, 288, 1356–1358. 10.1126/science.288.5470.1356
- Gehring, P.S., Tolley, K.A., Eckhardt, F.S., Townsend, T.M., Ziegler, T., Ratsoavina, F., Glaw, F. & Vences, M. (2012) Hiding deep in the trees: discovery of divergent mitochondrial lineages in Malagasy chameleons of the *Calumma nasutum* group. *Ecology and Evolution*, 2, 1468–79. 10.1002/ece3.269
- Giles, P.T. & Burgoyne, J.M. (2008) Skole, D.L. and Tucker, C.J. 1993: Tropical deforestation and habitat fragmentation in the Amazon: satellite data from 1978 to 1988. *Science* 260, pp. 1905--1910. *Progress in Physical Geography*, 32(5), 575–580.
- Glaw, F. & Vences, M. (2003) Amphibians. *In: Goodman, S.M. & Benstead, J.P. (Eds.) The Natural History of Madagascar*. The University of Chicago Press, Chicago, pp. 883–933.
- Glaw, F. & Vences, M. (2007) *A Field Guide to the Amphibians and Reptiles of Madagascar*. Vences & Glaw Verlags GbR, Köln, Germany, 496 pp.
- Haddad, N.M., Brudvig, L.A., Clobert, J., Davies, K.F., Gonzalez, A., Holt, R.D., Lovejoy, T.E., Sexton, J.O., Austin, M.P., Collins, C.D., Cook, W.M., Damschen, E.I., Ewers, R.M., Foster, B.L., Jenkins, C.N., King, A.J., Laurance, W.F., Levey, D.J., Margules, C.R., Melbourne, B.A., Nicholls, A.O., Orrock, J.L., Song, D.-X. & Townshend, J.R. (2015) Habitat fragmentation and

- its lasting impact on Earth's ecosystems. *Science Advances*, 1(2), e1500052. 10.1126/sciadv.1500052
- Harper, K.A., Macdonald, S.E., Burton, P.J., Chen, J., Brosnoff, K.D., Saunders, S.C., Euskirchen, E.S., Roberts, D.A.R., Jaiteh, M.S. & Esseen, P.-A. (2005) Edge Influence on Forest Structure and Composition in Fragmented Landscapes. *Conservation Biology*, 19(3), 768–782. 10.1111/j.1523-1739.2005.00045.x
- Harper, G.J., Steininger, M.K., Tucker, C.J., Juhn, D. & Hawkins, F. (2007) Fifty years of deforestation and forest fragmentation in Madagascar. *Environmental Conservation*, 34, 1–9. 10.1017/s0376892907004262
- Irwin, M.T., Wright, P.C., Birkinshaw, C., Fisher, B.L., Gardner, C.J., Glos, J., Goodman, S.M., Loiselle, P., Rabeson, P. & Raharison, J.-L. (2010) Patterns of species change in anthropogenically disturbed forests of Madagascar. *Biological Conservation*, 143, 2351–2362. 10.1016/j.biocon.2010.01.023
- Jenkins, R.K.B., Brady, L.D., Bisoa, M., Rabearivony, J. & Griffiths, R.A. (2003) Forest disturbance and river proximity influence chameleon abundance in Madagascar. *Biological Conservation*, 109, 407–415.
- Keenan, R.J., Reams, G.A., Achard, F., de Freitas, J.V., Grainger, A. & Lindquist, E. (2015) Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. *Forest Ecology and Management*, 352, 9–20. 10.1016/j.foreco.2015.06.014
- Laurance, W.F. (2004) Forest-climate interactions in fragmented tropical landscapes. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 359, 345–352. 10.1098/rstb.2003.1430
- Laurance, W.F., Delamonica, P., Laurance, S.G., Vasconcelos, H.L. & Lovejoy, T.E. (2000) Conservation: Rainforest fragmentation kills big trees. *Nature*, 404, 836–836.
- Laurance, W.F., Nascimento, H.E.M., Laurance, S.G., Andrade, A., Ewers, R.M., Harms, K.E., Luizão, R.C.C. & Ribeiro, J.E. (2007) Habitat Fragmentation, Variable Edge Effects, and the Landscape-Divergence Hypothesis. *PLoS ONE*, 2, e1017. 10.1371/journal.pone.0001017
- Lehman, S.M., Rajaonson, A. & Day, S. (2006) Edge effects and their influence on lemur density and distribution in southeast Madagascar. *American Journal of Physical Anthropology*, 129(2), 232–241.
- Lehtinen, R.M. (2002). The use of screw pines (*Pandanus* spp.) by amphibians and reptiles in Madagascar. *Herpetological Bulletin*, 82, 20–25.
- Lehtinen, R.M., Ramanamanhato, J.-B. & Raveloarison, J.G. (2003) Edge effects and extinction proneness in a herpetofauna from Madagascar. *Biodiversity and Conservation*, 12, 1357–1370.
- Malcolm, J.R. (1994) Edge Effects in Central Amazonian Forest Fragments. *Ecology*, 75(8), 2438–2445.
- Matlack, G.R. (1993) Microenvironment variation within and among forest edge sites in the eastern United States. *Biological Conservation*, 66, 185–194.
- Matlack, G.R. (1994) Vegetation Dynamics of the Forest Edge — Trends in Space and Successional Time. *Journal of Ecology*, 82(1), 113–123.
- Murcia, C. (1995) Edge effects in fragmented forests: Implications for conservation. *Trends in Ecology and Evolution*, 10(2), 58–62.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858.
- Nagy, Z.T., Sonet, G., Glaw, F. & Vences, M. (2012) First large-scale DNA barcoding assessment of reptiles in the biodiversity hotspot of Madagascar, based on newly designed COI primers. *PLoS ONE*, 7, e34506. 10.1371/journal.pone.0034506

- Ndriantsoa, S.H., Randrianiaina, R.-D., Ravoahangimalala Ramilijaona, O., Vences, M. & Glos, J. (2012) Description of two tadpoles of Malagasy treefrogs, *Spinomantis* sp. aff. *peraccae* and *Spinomantis tavaratra* (Anura: Mantellidae). *Zootaxa*, 3248, 66–68.
- Perl, R.G.B., Nagy, Z.T., Sonet, G., Glaw, F., Wollenberg, K.C. & Vences, M. (2014) DNA barcoding Madagascar's amphibian fauna. *Amphibia-Reptilia*, 35, 197–206.
- Pickett, S.T. & White, P.S. (2013) The ecology of natural disturbance and patch dynamics. Academic Press, MA, USA, 472 pp.
- R Development Core Team. (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rabearivony, J., Raselimanana, A.P., Andriamazava, M.A., Thorstrom, R. & de Roland, L.-A.R. (2010) A new locality for the endangered microhylid frog *Scaphiophryne boribory* from northern Madagascar and a rapid survey of other amphibians of the Bemanevika region. *Herpetology Notes*, 3, 105-109.
- Rabearivony, J., Rasamoelina, M., Raveloson, J., Rakotomanana, H., Raselimanana, A.P., Raminosoa, N.R. & Zaonarivelo, J.R. (2015) Roles of a forest corridor between Marojejy, Anjanaharibe-Sud and Tsaratanana protected areas, northern Madagascar, in maintaining endemic and threatened Malagasy taxa. *Madagascar Conservation & Development*, 10, 85–92. 10.4314/mcd.v10i2.7
- Rakotomalala, D. (2002) Diversité des reptiles et amphibiens de la Réserve Spéciale de Manongarivo, Madagascar. *In: Gautier, L. & Goodman, S.M. (Eds.) Inventaire Floristique et Faunistique de la Réserve Spéciale de Manongarivo (NW Madagascar)*. Conservatoire et Jardin Botaniques de Genève, Geneva, pp. 340–358.
- Rakotondravony, H.A. & Goodman, S.M. (2011) Rapid herpetofaunal surveys within five isolated forests on sedimentary rock in western Madagascar. *Herpetological Conservation and Biology*, 6(2), 297–311.
- Ramanamanjato, J.-B. & Lehtinen, R. (2006) Effects of rainforest fragmentation and correlates of local extinction in a herpetofauna from Madagascar. *Applied Herpetology*, 3, 95–110.
- Raxworthy, C.J. & Nussbaum, R.A. (1996) Montane Amphibian and Reptile Communities in Madagascar. *Conservation Biology*, 10, 750-756.
- Riemann, J.C., Ndriantsoa, S.H., Raminosoa, N.R., Rödel, M.-O. & Glos, J. (2015) The value of forest fragments for maintaining amphibian diversity in Madagascar. *Biological Conservation*, 191, 707–715. 10.1016/j.biocon.2015.08.020
- Ries, L., Fletcher, R.J., Battin, J. & Sisk, T.D. (2004) Ecological Responses to Habitat Edges: Mechanisms, Models, and Variability Explained. *Annual Review of Ecology, Evolution, and Systematics*, 35, 491-522. 10.1146/annurev.ecolsys.35.112202.130148
- Riitters, K., Wickham, R., O'Neill, B., Jones, B. & Smith E. (2000). Global-scale patterns of forest fragmentation. *Conservation Ecology*, 4(2), 3.
- Saunders, D.A., Hobbs, R.J. & Margules, C.R. (1991) Biological Consequences of Ecosystem Fragmentation — a Review. *Conservation Biology*, 5(1), 18–32.
- Vajda, V., Raine, J.I. & Hollis, C.J. (2001) Indication of Global Deforestation at the Cretaceous-Tertiary Boundary by New Zealand Fern Spike. *Science*, 294, 1700-1702. 10.1126/science.1064706
- Vences, M., Glaw, F., Andreone, F., Jesu, R. & Schimmenti, G. (2002) Systematic revision of the enigmatic Malagasy broad-headed frogs (*Laurentomantis* Dubois, 1980), and their phylogenetic position within the endemic mantellid radiation of Madagascar. *Contributions to Zoology*, 70, 191–212.
- Vetter, D., Rüker, G. & Storch, I. (2013) A meta-analysis of tropical forest edge effects on bird nest predation risk: Edge effects in avian nest predation. *Biological Conservation*, 159, 382–395.

- Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F. & Vences, M. (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences*, 106, 8267–8272. 10.1073/pnas.0810821106
- Wade, T.G. et al. (2003) Distribution and causes of global forest fragmentation. *Ecology and Society*, 7(2), 7.
- Whitmore, T.C., Laurance, W.F. & Bierregaard, R.O. (1997) *Tropical forest remnants: ecology, management, and conservation of fragmented communities*. University of Chicago Press, IL, USA, 632 pp.
- Whitmore, T.C. (1998) *An introduction to tropical rain forests*. Oxford University Press, Oxford, UK, 296 pp.
- Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*. Springer, NY, USA, 213 pp.
- Wollenberg, K.C., Vieites, D.R., van der Meijden, A., Glaw, F., Cannatella, D.C. & Vences, M. (2008) Patterns of endemism and species richness in Malagasy cophyline frogs support a key role of mountainous areas for speciation. *Evolution*, 62, 1890–907. 10.1111/j.1558-5646.2008.00420.x
- Wollenberg, K.C., Vieites, D.R., Glaw, F. & Vences, M. (2011) Speciation in little: the role of range and body size in the diversification of Malagasy mantellid frogs. *BMC Evolutionary Biology*, 11, 217. 10.1186/1471-2148-11-217
- Wollenberg, K.C., Glaw, F. & Vences, M. (2012) Revision of the little brown frogs in the *Gephyromantis decaryi* complex with description of a new species. *Zootaxa*, 3421, 32-60.
- Wright, S.J., 2005. Tropical forests in a changing environment. *Trends in Ecology and Evolution*, 20(10), 553–560.