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Ecological Correlates for Endemic and Threatened Amphibians in the Uluguru Nature Forest Reserve, Tanzania

Joseph M. Christopher*, Wilirk Ngalason and John V. Lyakurwa
Department of Zoology and Wildlife Conservation, University of Dar es Salaam, P. O. Box 35064, Dar es Salaam, Tanzania.
*****Corresponding author, email: christopherjoseph635@gmail.com*
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Abstract

Information on habitat characteristics for endemic and threatened amphibians along the altitudinal gradient in the Uluguru Nature Forest Reserve (UNFR) is limited. We assessed habitat characteristics associated with the occurrence of endemic and threatened amphibians in the Uluguru Nature Forest Reserve between April and June 2022. Bucket pitfall traps, time-constrained audio-visual surveys, plots and opportunistic searching were used to study amphibians in 6 sites along elevational gradient from 600 m to 2100 m a.s.l. Eighteen amphibian species were recorded, thirteen of them being endemic to the Eastern Arc Mountains, two near-endemic and three widely distributed. Among the species recorded, seven are categorized as threatened as per IUCN Red List. We observed more endemic and threatened amphibians in the submontane forests (1200–1800 m) than in other sites. Negative correlations existed between temperature, canopy cover, leaf litter cover and leaf litter depth and species richness, whereas positive correlations existed between humidity and species richness. No single factor was responsible for the occurrence of endemic and threatened amphibians in the Uluguru Nature Forest Reserve. This is only a snapshot survey on the occurrence of threatened and endemic amphibians and their associated habitat features in the northern part of the Uluguru Nature Forest Reserve. A long-term monitoring of these amphibian species is recommended especially at this era of unprecedented forest loss and degradation.

Keywords: Eastern Arc Mountains, Forests, Biodiversity, Conservation.

Introduction

Amphibians are the most threatened vertebrates on Earth with more than 35% of the species assessed by the IUCN considered to be at risk of extinction (IUCN 2022). Habitat alternation, especially through logging, firewood and building poles collection, uncontrolled fires, and clearance for subsistence and cash–crop cultivation are the main drivers for amphibian species loss (Hof et al. 2011, Menegon et al. 2020). Other key threats include the influence of climate change, pollution, overharvest, and fungal and parasitic infections (Hof et al. 2011).

The Eastern Arc Mountains (EAMs) are renowned for harbouring a large number of endemic and threatened species of animals and plants (Burgess et al. 2007, Gereau et al. 2016). They hold the highest concentration of vertebrates per 100 km^2 than any other biodiversity hotspot (Myers et al. 2000). Uluguru Mountains form one of the most important blocks in the EAMs (Burgess et al. 2007, Gereau et al. 2016). The Uluguru mountain block has several forests under varying levels of protection, with the recently established Uluguru Nature Forest Reserve (UNFR) being the largest forest patch in this

block (TFCG 2017, Mkonyi 2021). At least 73 amphibian species are endemic to the EAMs of Tanzania (Menegon et al. 2020), 32 of them occurring in the UNFR, five of which are endemic (Menegon et al. 2020, Frost 2023). In addition, 12 of the 32 species occurring in the UNFR are classified as threatened by the IUCN Red List (IUCN 2022). Despite the remarkable statistics about the biodiversity of the EAMs, the forests are among the most threatened ecosystems (Hall et al. 2009) particularly due to logging, firewood collection, bush fires and agriculture (Hamunyela et al. 2020, Menegon et al. 2020, Liedtke et al. 2022) even though a large portion of the forests fall under the protected area network of Tanzania. The current area of the EAMs represents less than 30% of the original forest (Hall et al. 2009) with the remaining patches showing loss of quality through poles cutting, local/small scale mining and understorey clearing for spice cultivation. These activities reduce the forest quality for amphibians and are hard to detect through satellite imagery. The Uluguru mountain block is not exempted from these threats (Burgess et al. 2002, Doggart et al. 2004, Hamunyela et al. 2020, Kilawe et al. 2021).

The history of herpetological studies in the UNFR is long, dating back from 1920s to very recent, e.g., Loveridge (1925), Doggart et al. (2004), Mkonyi (2005), Ngalason (2005), Ngalason and Mkonyi (2011) and Mkonyi (2021). Most of these studies were mainly explorative surveys resulting to checklists and descriptions of new species with only brief accounts on habitat characteristics. Mkonyi (2005), Ngalason (2005), Ngalason and Mkonyi (2011) provided some details on reproductive biology and altitudinal distribution of amphibians in the southern part of the UNFR (former Uluguru South Forest Reserve). South portion of the UNFR contains mosaics of habitats including forests and grassland patches on the plateau while Uluguru north is mostly a continuous forest stretching from about 700 m to 2170 m a.s.l. (Lovett and Pócs 1993). This study sought to assess the association between canopy cover, leaf litter cover and depth, temperature and humidity and the occurrences of endemic and threatened amphibian species along the elevation gradient in the northern part of Uluguru Nature Forest Reserve, Morogoro, Tanzania.

Materials and Methods

Study area

This study was conducted in the Uluguru Nature Forest Reserve, in the central part of the Eastern Arc Mountains (Figure 1). The Uluguru Nature Forest Reserve was gazetted in 2008 by the amalgamation of the former Uluguru North, Uluguru South, Bunduki I and II forest reserves, and Bunduki 'gap' corridor (TFCG 2017). The study was conducted on the northern part of the nature reserve, covering what used to constitute the Uluguru North Forest Reserve, that lies between 6° 51'–7° 01' S and 37° 37'–37° 45' E (Doggart et al. 2004). Uluguru Nature Forest Reserve receives short rains from October to December and long rains from March to May (Lovett and Pócs 1993). Estimated rainfall is between 1200 and 3100 mm/year on the western slopes and between 2900 and 4000 mm/year on the eastern slopes (Doggart et al. 2004). Higher altitudes are considerably cooler, with temperature dropping to -7 °C during the cold seasons between June and August (Lovett and Pócs 1993). On the lowlands temperature ranges between 21 °C in July and 34 °C in December (MNRT 2016). The northern part of Uluguru Nature Forest Reserve is mostly characterised by moist forests which can be categorised as submontane (500–1500 m), montane (1500–1850 m) and upper montane forests (1850–2400 m), whereas the southern part is more complex, having moist forests that surround the upland grassland, swamps and forest patches of the Lukwangule plateau (Lovett and Pócs 1993).

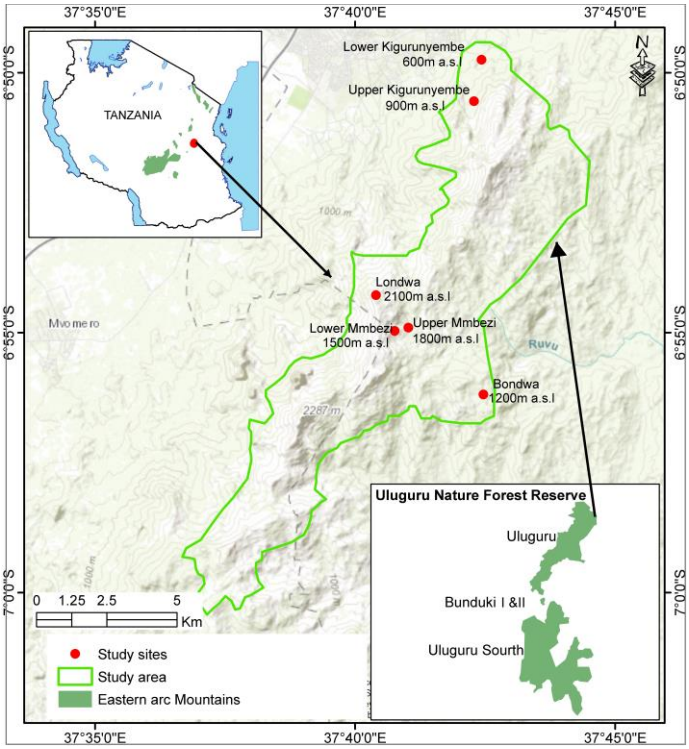

Figure 1: Location of study sites in the northern part of the Uluguru Nature Forest Reserve,Tanzania. Note; The insert map shows the location of the study area in the Eastern Arc Mountains of Tanzania (top left) and Uluguru Nature Forest Reserve (bottom right).

Data collection

Data were collected during the long rainy season, between the end of April and mid–June 2022. Six sites were established at an interval of 300 m a.s.l. (at 600 m, 900 m, 1200 m, 1500 m, 1800 m and 2100 m a.s.l.). Methods that were used to study amphibians include a drift fence with bucket pitfall traps, time constrained audio–visual surveys, plots and opportunistic searching. Two bucket pitfall trap lines with drift fences were set at each site. Each trapline consisted of seven plastic buckets of 20 litres set in a 'Y' shape following McDiarmid et al. (2012). The traps were checked twice a day, early in the morning and at noon following Lyakurwa et al. (2019) for seven consecutive nights per site, making a total of 98 trap nights per site and 588 trap nights for the whole study. Additionally, nine plots of 5 m x 5 m each were established at the end of each bucket pitfall trapline, 5 m from the last bucket (three plots at each Y–arm). The plots were set at an interval of 5 m. Four people searched thoroughly for amphibians at each plot (each person starting at the corner of the plot) at night (starting from 19:30 hrs) by overturning litter and logs, and on tree trunks until the whole plot was covered.

During time constrained audio–visual surveys, each study site was searched for amphibians by four people in the morning between 06:30 and 08:30 hrs, and at night between 19:30 and 21:30 hrs for 5 consecutive days, making a total of 80 person–hours per site and 480 person–hours for the whole study. All amphibians encountered casually within the study area were recorded as opportunistic encounters. Habitat characteristics were measured in four cardinal directions at each study site and then averaged, at all points where amphibians were encountered following Lyakurwa (2019). All amphibians obtained were

identified in the field by using Channing and Rödel (2019) and Menegon et al. (2020) and representative voucher specimens were preserved and taken to the University of Dar es Salaam for confirmation of identity and storage for future reference. Species names followed Frost (2023) whereas threat status followed IUCN (2022).

Data analysis

Rarefaction curves (McDiarmid et al. 2012) for the six sites were plotted to investigate the adequacy of the sampling effort. Shannon–Wiener diversity index (*H'*) was used for species diversity which was then compared among the six elevation zones using Chi–square test (Zar 2010). To test if the same species were found across the six elevation zones, we employed the Bray–Curtis similarity index (Greenacre and Primicerio 2013). Generalized linear model was used to examine how species were distributed along the elevation gradient, whereby linear, exponential, logarithmic and polynomial fits were tested to determine the model which best explains the relationship by examining the resultant residual sum of squares following Kutt et al. (2011). Canonical correspondence analysis (ter-Braak 1986) was performed to assess the relationship between habitat characteristics and abundance of endemic and threatened amphibian species at different elevations. All statistical analyses were performed in R–Software version 4.2.2 (R Core Team 2022) and Paleontological Statistics Software (PAST) version 3.21. Comparisons were considered significant when p–value was less than or equal to 0.05.

Results

A total of 659 individuals belonging to 18 amphibian species (16 anurans and two caecilians), from 10 families were recorded (Table 1). Generally, more individuals were found at low elevations and decreased in higher elevations, including 161 individuals at 900 m, 157 individuals at 600 m, 86 at 1200 m, 89 at 1500 m, 115 at 1800 and 51 individuals at 2100 m. However, only few individuals of widely distributed species dominated the low elevations, e.g. at 600 m, 100 individuals were represented only by *Arthroleptis stenodactylus and* 113 individuals of *Arthroleptis xenodactyloides* at 900 m. Three species were only recorded once (singletons) including *Afrixalus uluguruensis*, *Boulengelura uluguruensis* and *Hoplophryne uluguruensis*, two were doubletons (*Leptopelis flavomaculatus* and *Scolecomorphus kirkii*) and the rest were recorded at least thrice. Thirteen species are endemic to the EAMs, three of them being endemic to Uluguru Mountains (Table 1). Seven species are considered threatened as per the IUCN red list (Table 1). Only one species (*Arthroleptis xenodactyloides*) was found throughout the elevational ranges from 600 m to 2100 m and *A. affinis* was found in four of the sites from 1200 m to 2100 m. Also, four species were found in three of the sites including *Leptopelis parkeri* and *Callulina creffti* between 1200 m and 1800 m and *Nectophrynoides pseudotornieri* and *N. viviparus* were found at 1500 m and 2100 m (Table 1). One species *N. pseudotornieri* represents a range extension from previously known range of below 1500 m (Menegon et al. 2020, Frost 2023). Most species were recorded at 1200 m and the lowest number at 900 m. The sites at 600 m, 1500 m and 1800 m displayed the same number of species (Table 1). There was no significant difference in species diversity indices between study sites (χ^2= 0.3, p = 0.99). The distributional pattern of endemic species corresponds to that of threatened species (Table 1) with mid and higher elevations having more species than the lower elevations.

Table 1: Amphibian species richness at different elevations in the northern part of the Uluguru Nature Forest Reserve, Tanzania

Family/Species/Common name	Altitude (m)						IUCN status	Endemism
	600	900	1200	1500	1800	2100		
Family Arthroleptidae								
Arthroleptis affinis (Related squeaker)	–	–	x	x	x	x	LC	EAMs
Arthroleptis stenodactylus (Long–fingered squeaker)	x	x	–	–	–	–	LC	W
Arthroleptis xenodactyloides (Dwarf squeaker)	x	x	x	x	x	x	LC	W
Leptopelis flavomaculatus (Yellow–spotted tree frog)	x	–	–	–	–	–	LC	W
Leptopelis parkeri (Parker's forest tree frog)	–	–	x	x	x	–	EN	EAMs
Leptopelis uluguruensis (Uluguru forest tree frog)	–	–	x	–	–	–	NT	EAMs
Family Brevicipitidae								
Callulina kreffti (Krefft's secret frog)	–	–	x	x	x	–	LC	EAMs
Family Bufonidae								
Nectophrynoides tornieri (Tornier's tree frog)	–	–	x	–	x	–	LC	EAMs
Nectophrynoides pseudotornieri (False Tornier's Forest toad)	–	–	–	x	x	x	CR	EAMs
Nectophrynoides viviparus (Robust viviparus toad)	–	–	–	x	x	x	LC	EAMs
Family Hyperoliidae								
Afrixalus uluguruensis (Uluguru banana frog)	–	–	x	–	–	–	VU	EAMs
Family Microhylidae								
Hoplophryne uluguruensis (Uluguru Three–fingered frog)	–	–	–	–	–	x	EN	EAMs
Family Petropedetidae								
Arthroleptides yakusini (Southern montane torrent frog)	x	x	–	–	–	–	EN	EAMs
Family Ptychadenidae								
Ptychadena anchietae (Plain grass frog)	x		–	–	–	–	LC	W
Family Pyxicephalidae								
Amietia tenuoplicata (Amani River frog)	x	x	–	–	–	–	LC	NE
Family Herpelidae								
Boulengelura uluguruensis (Uluguru pink caecilian)	–	x	–	–	–	–	LC	EAMs
Family Scolecomorphidae								
Scolecomorphus kirkii (Kirk's caecilian)	x	–	x	–	–	–	LC	NE
Scolecomorphus uluguruensis (Uluguru caecilian)	–	–	–	x	–	x	EN	EAMs
Total number of species	7	5	8	7	7	6		
Shannon index value (H')	1.08	0.85	1.66	1.17	1.25	1.32		
Total number of endemic species	2	3	7	7	7	6		
Total number of threatened species	1	1	4	2	3	2		

Note: CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Near Threatened, LC = Least Concern, EAMs = Endemic to Eastern Arc Mountains of Tanzania, NE = Near Endemic (found mainly in Tanzania, but a small population extends to one or two neighbouring countries) and W = Widely distributed.

Bray–Curtis similarity index showed sites adjacent to each other to be more similar in species composition than distant ones. Sites that had high species similarity include 600 m vs 900 m (0.71), 1200 m vs 1500 m (0.61) and 1800 m vs 2100 m (0.58) (Figure 2, Table 2). The lower elevation sites possessed more widely distributed (generalist) species, e.g. *Arthroleptis stenodactylus* and *Ptychadena anchietae* than the higher elevations, whereas most habitat specialists, e.g. *Nectophrynoides pseudotornieri* and *N. viviparus* were restricted to the mid and higher elevations (Table 1). Generally, the relationship between endemic and threatened amphibian species occurrences and elevation followed a polynomial distribution $y = -5E–06x^2+0.17x–6.8286$, $R^2 = 0.8951$ (Figure 3). However, species rarefaction curves did not reach asymptote indicating the chances of getting more species with more sampling efforts (Figure 4).

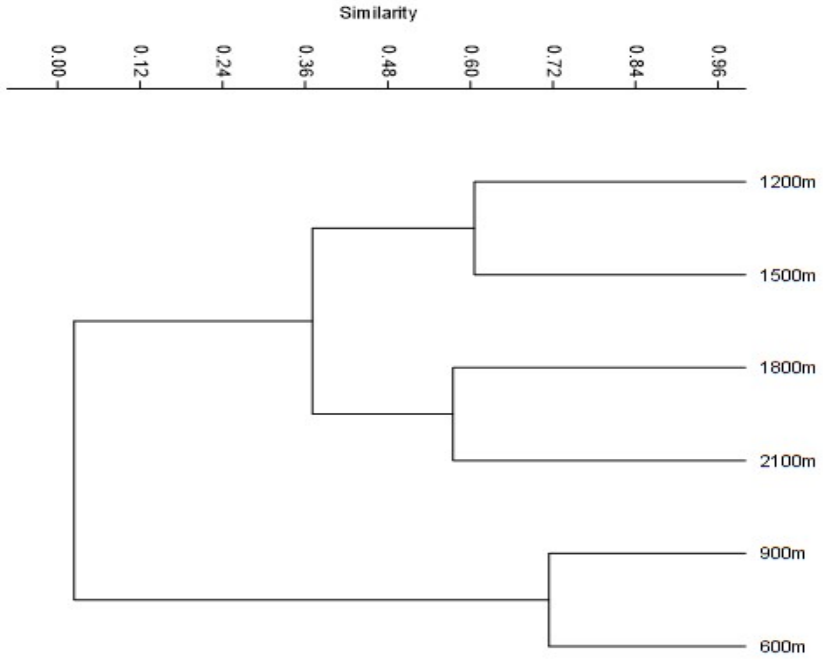

Figure 2: Similarity cluster for endemic and threatened amphibians among the six elevation zones in the Uluguru Nature Forest Reserve based on Bray–Curtis similarity index (Single Average Link).

Table 2: Bray Curtis species similarity matrix of endemic and threatened amphibians for the 6 sites surveyed in the northern part of the Uluguru Nature Reserve, Tanzania from April 2022 to mid–June 2022

	600 m	900 m	1200 m	1500 m	1800 m	2100 m
600 m	1.00					
900 m	**0.71**	1.00				
1200 m	0.02	0.00	1.00			
1500 m	0.00	0.00	**0.61**	1.00		
1800 m	0.00	0.00	0.36	0.36	1.00	
2100 m	0.00	0.00	0.27	0.37	**0.58**	1.00

Note; 0 represents 100% dissimilarity whereas 1 represents 100% similarity. Numbers in bold black indicate the most strongly related sites (>50%).

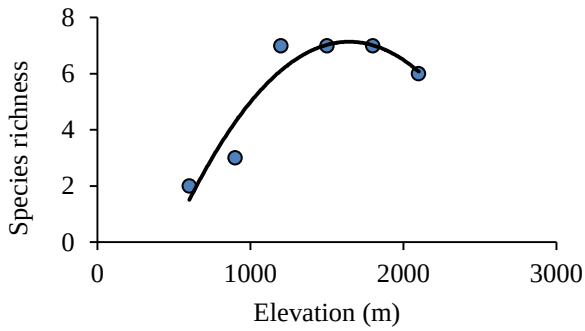

Figure 3: Relationship between elevation and species richness of endemic and threatened amphibian species in the northern part of Uluguru Nature Forest Reserve, Tanzania.

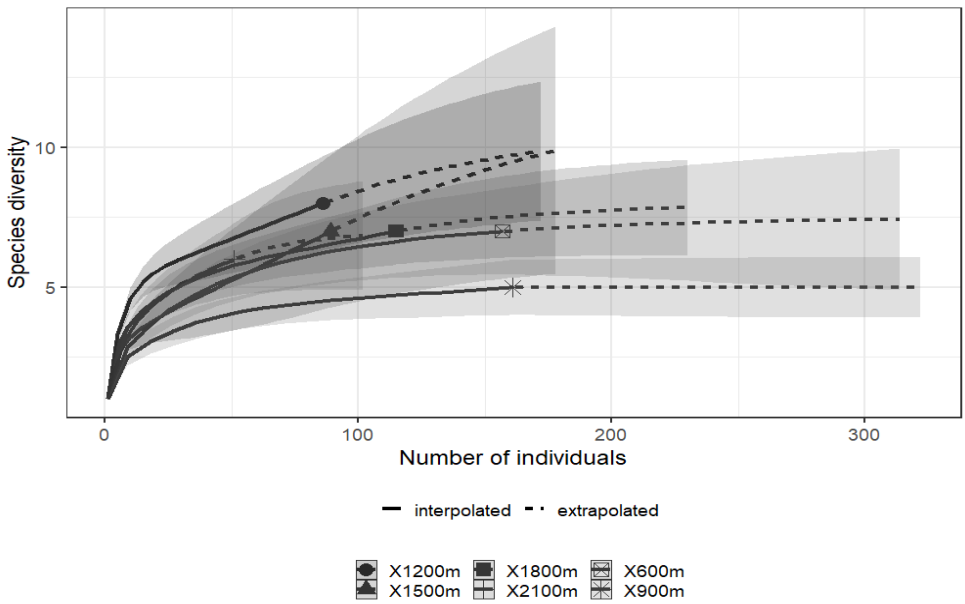

Figure 4: Rarefaction curves for amphibian species recorded in the six sampled elevation zones from April 2022 to mid–June 2022 at the northern part of the Uluguru Nature Forest Reserve, Tanzania. Shaded region surrounding each line represents 95% confidence levels. The numbers in the key indicate elevation (m).

In assessing the association between habitat characteristics and amphibian species richness and abundance the first two ordination axes of the CCA biplot explained 58.29% and 37.81% of the variance in the species dataset with eigenvalues of 0.535 and 0.347, respectively. Axis 1, which accounted for 58.29% of the variance, successfully showed the relationship between habitat characteristics and the relative abundance of endemic and threatened amphibian species. Endemic and threatened amphibian species with the strongest positive association with Axis 1 were *Arthroleptides yakusini* and *Boulengerula uluguruensis* (Figure 5). Also, temperature, percentage canopy cover, leaf litter cover and depth showed positive association with Axis 1. Furthermore, *Hoplophryne uluguruensis*, *Scolecomorphus uluguruensis*, *Nectophrynoides pseudotornieri*, *Nectophrynoides viviparus*, *Arthroleptis affinis*, *Callulina kreffti*,

Nectophrynoides tornieri, Leptopelis parkeri, Afrixalus uluguruensis and *Leptopelis uluguruensis* were negatively associated with Axis 1 and relative humidity was negatively associated with axis 1. The endemic and threatened amphibian species with strong association with leaf litter including *Boulengerula uluguruensis* dominated the right portion of the plot. This portion was mostly influenced by temperature, leaf litter depth, canopy cover and leaf litter cover. Endemic and threatened species that are typically arboreal including *Nectophrynoides pseudotornieri, Callulina kreffti, Leptopelis parkeri, Afrixalus uluguruensis* and *Leptopelis uluguruensis* fall on the left portion of the plot which is strongly defined by high relative humidity (Figure 5).

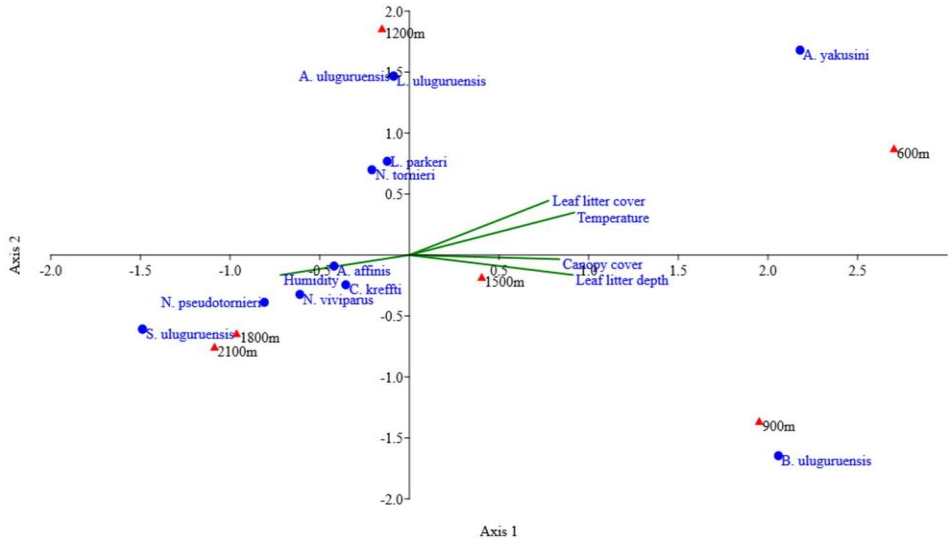

Figure 5: Ordination diagram of the association between habitat characteristics and relative abundance of endemic and threatened amphibian species in the northern part of the Uluguru Nature Forest Reserve, Tanzania. Note: habitat characteristics are represented by green lines and amphibian species are represented by blue dots. Sites are represented by red triangles.

Discussion

This study provides results of an assessment of the distribution of thirteen threatened and endemic amphibian species in the northern part of Uluguru Nature Forest Reserve, an amphibian hotspot that has suffered a significant forest loss in the previous decades (Hamunyela et al. 2020, Menegon et al. 2020). Endemic and threatened amphibian species were observed mostly in higher elevations compared to the lowland forest. This pattern is in agreement with Menegon and Salvidio (2005) and Burgess et al. (2002) who reported the same trend in Uzungwa Scarp Nature Forest Reserve and Eastern Arc Mountains, respectively. The studies indicated most of the endemic and threatened amphibian species to be concentrated on submontane forest areas (between 500 and 1500 m). Mid elevations are characterised by intermediate environmental conditions which allow both the low and high elevation species to exist (McCain 2010), contributing to a relative higher number of the endemic and threatened species in these zones. Similar results have been presented by Lyakurwa et al. (2019) who recorded more endemic and threatened reptiles in the submontane zone (between 800 and 1400 m). The pattern observed for the endemic and threatened species is in contrast to that of all amphibians observed by Mkonyi

(2005), Ngalason and Mkonyi (2011) in the southern part of the Uluguru Nature Forest Reserve which showed the decrease in number of species with an increase in elevation. Navas (2002) indicated higher elevations to have extreme environmental conditions that require special adaptations for animals to survive. The occurrence of more endemic species at higher elevations could be explained by the landscape heterogeneity together with the historic adaptation and diversification of species in these mountains (Menegon 2015). Several studies have indicated mid-elevation peaks or what is considered a hump–shaped relationship between species diversity and elevation, e.g., Paudel et al. (2018). The occurrence of more endemic and threatened species at higher elevations in the northern part of the Uluguru Nature Forest Reserve can also be related to the height of the mountain which is not very high as compared to other mountains such as Kilimanjaro in Tanzania.

Sites that were adjacent to each other had higher amphibian species similarity than distant ones, which agree well with the findings of Ngalason and Mkonyi (2011) in the southern part of the Uluguru Nature Forest Reserve. The same pattern has been observed for amphibians and reptiles in other Eastern Arc Mountains including Uzungwa Scarp Nature Forest Reserve (Menegon and Salvidio 2005, Lyakurwa et al. 2019). The observations could be associated with microhabitat characteristics that tend to be relatively more similar in adjacent than distant sites.

In this study, canopy cover decreased with elevation, although greater species richness of endemic and threatened amphibians was observed at relatively higher elevations. This is in contrast to Villacampa et al. (2019) who reported amphibian species richness to be higher in elevations with high canopy cover. Canopy cover is a strong predicator of amphibian community composition and abundance as it regulates relative humidity, provides shade and leaf litter to cover the ground for leaf litter frogs (Burrow and Maerz 2022). However, a lot of logging and pole cutting were observed in the sampled sites and these might have affected the findings. Furthermore, the study observed a decrease in leaf litter cover and depth with elevation. Submontane (between 500 and 1500 m a.s.l.) and montane forests (between 1500 and 1850 m a.s.l.) had more leaf litter cover and depth than the upper montane forests. Urbina–Cardona et al. (2006) reported a high correlation between leaf litter cover and depth and richness of leaf–litter amphibian species. This study found more leaf litter amphibian species in the lower elevations than in the higher elevations (Table 1) corresponding to the leaf litter cover and depth pattern. Sites with deep leaf litter generally sustain high numbers of amphibian species because leaf litter is an important source of resources and nutrient for amphibians (Magee 2019).

An inverse relationship was recorded between air temperature and the richness of endemic and threatened amphibian species on the Uluguru North Mountain. These findings are in contrast to the general pattern of the decrease of amphibian species richness with an increase in elevation which is normally associated with a decrease in temperature. Pizarro and Moreno–Rueda (2009) reported a positive correlation between temperature and amphibian species richness, where the richness of amphibian species increased with temperature. Similarly, Gillman and Wright (2014) reported an increase in amphibian species with a decrease in temperature. The endemic and threatened amphibians on the Uluguru Mountain were mostly found at relatively higher elevations reflecting their adaptation to such conditions. The results obtained could be explained by the nature and the location of the mountain which is relatively low and located in the tropics. The highest mountains such as Mount Kilimanjaro display a pattern of the decrease of richness with elevation (Zancolli et al. 2014). Different amphibian species are adapted to different conditions including high elevation species that are adapted to live in cooler areas and lowland species that are found on relatively warm areas (Muths et al. 2017). In addition, a positive correlation was observed between relative humidity and

richness of endemic and threatened amphibian species in the Uluguru Nature Forest Reserve. These findings concur to those of Cabrera–Guzman and Reynoso (2012) and da Silva et al. (2012) who reported a high number of amphibian species in areas with higher relative humidity. Amphibians have moist, thin and permeable skins that make them susceptible to evaporative water loss thus they tend to restrict themselves to areas with relatively higher humidity levels (da Silva et al. 2012).

Nine amphibian species known to occur in the Uluguru mountains (Frost 2023) were not detected during this study. Some of these species are restricted to the southern part of the reserve, e.g., *Nectophrynoides cryptus* and *N. laevis* and others might have been missed due to their secretive habits, e.g., *Probreviceps* species and *Spelaeophryne mentheri* which spend most of their time underground. Some species may also occur in low numbers and thus not easy to be detected in a single survey. This is supported by the species rarefaction curves which did not reach asymptote. Decreasing detectability for some species has been reported from other localities within the EAMs (Liedtke et al. 2022) and this might also be connected to the ongoing habitat destructions. Some species were recorded only once and others, though known to occur widely across the elevation range were missed in a number of sites. A long–term monitoring programme will provide a more complete pattern of amphibians and their associated habitats in the Uluguru mountains especially for species that were represented by very few individuals or missed in some elevations. Similarly, some species, e.g., *Nectophrynoides* spp, *Arthroleptis affinis*, *Callulina kreffti*, *Arthroleptis xenodactyloides* and *L. parkeri* represent species complexes (Menegon et al. 2020) of which a clear habitat association will be revealed when these taxonomic problems are resolved. Nevertheless, this study provides a general overview of amphibian species habitats in a highly threatened amphibian hotspot and can be used to predict the effect of the ongoing deforestation.

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