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Title: Investigating the Dietary Composition of two Critically Endangered Frogs from Tanzania: Disseminating Conservation Education Amidst the Ongoing Forest Loss.

1. Background

Despite the ecological and conservation significance of Mkingu Nature Forest Reserve (MNFR) and its critical role in preserving the unique biodiversity of the Eastern Arc Mountains, the reserve faces significant risks from human activities, particularly the conversion of forest to cropland through illegal clearing of vegetation for cardamom and cocoa plantation, illegal logging, and wildfires (Doggart et al. 2007, Hamunyela et al. 2020). As per the Adding Value to the Arc project (AVA) examination of deforestation within the South Nguru landscape, around 475 hectares of forest were cleared within the Mkingu Nature Forest Reserve's boundaries from October 2010 to January 2014 (Lyimo 2014). Similarly, Hamunyela et al (2020) reported that approximately 20,487 hectares of forest land, accounting for 27% of the total benchmark forest area of 75,735 hectares, underwent deforestation (14,341 hectares) and degradation (6,146 hectares) in the period 2001 to 2017 in Mvomero district where the reserve is situated.

The ongoing surveys on forest toads, *Nectophrynoides spp* in the same locality where I led the team recorded newly cleared patches inside the forest, implying that the current rate of forest loss appears to be even greater (Pers. obs) (Figure 1). Most of these clearings affect the understorey vegetation and are not detected by satellite images, leading to possible underestimation of the destructions (Truscott 2023). This challenge facing the reserve results in habitat loss for the species inhabiting it including the two targeted species. The integrity of habitat directly influences their ability to find food, shelter, and suitable breeding sites, ultimately impacting their population dynamics and long-term survival prospects (Hollander et al. 2013).

Amphibians are one of the species groups that most of their species depend on the climatic conditions found in forests (Greenberg et al. 2018). The loss of these forests leads to habitat loss, particularly for forest-dependent species like amphibians. This clarifies why amphibian species face a higher extinction rate than other taxa (Bishop et al. 2012), with habitat loss being recognized as the primary contributing factor (Chanson, J. et al. 2008).

Callulina hanseni (Figure 2) and *Callulina meteora* (Figure 3) are frogs that rely on forest habitats and are endemic to the higher elevations of the MNFR (Menegon et al. 2020). These two species coexist in the same habitat, occurring in less than 100 km² of the total area, making them critically endangered according to the IUCN (Loader et al. 2010ab, Menegon and Gower 2011). The ongoing forest loss might lead to the extinction of the two species, this raises alarm for urgent conservation efforts. The tragic example of the *Nectophrynoides asperginis* (Kihansi spray toad) in Tanzania, which became extinct in the wild mainly due to habitat alteration

caused by the construction of an electric dam serves as a reminder of the potential consequences (Nahonyo et al. 2017).

The critical endangerment of *Callulina hanseni* and *Callulina meteora*, two frog species endemic to the Mkingu Nature Forest Reserve (MNFR), underscores the urgent need to understand the factors affecting their survival. Amidst the ongoing rapid forest loss threatening their habitat, there is a significant knowledge gap regarding their basic ecological characteristics, particularly their feeding habits. It is worth noting that there are only two studies on the two species *C. hanseni* by Loader et al. (2010ab) and *C. meteora* by Menegon and Gower (2011) which primarily focused on their taxonomic identification. The specific dietary preferences of these frogs remain poorly understood. This lack of information hinders conservation efforts aimed at preserving their habitat and addressing the impacts of anthropogenic activities on their food availability within the forest. Therefore, the problem addressed in this study is to investigate the food composition of *Callulina hanseni* and *Callulina meteora*, elucidating their dietary requirements and the potential implications of habitat degradation on their feeding ecology.

Despite the awareness efforts such as the Adding Value to the Arc project (AVA) (Lyimo 2014) and the ongoing forest reforestation project by PAMS Foundation in some of the villages adjacent to a reserve neither of which focused on amphibians, there remains a lack of awareness among local communities regarding the amphibian species found within the forest, including the two targeted species.

Consequently, the proposed research seeks to investigate the dietary preferences of the two species, recognizing it as a key ecological aspect influenced by habitat quality. Furthermore, the study aims to promote awareness about the significance of conserving forest biodiversity including amphibians, particularly emphasizing the importance of preserving the habitat of these two species.



Figure 1: A picture showing a cleared vegetation patch inside the Mkingu Nature Forest Reserve boundaries



Figure 2: *Callulina hanseni* in Mkingu Nature Forest Reserve



Figure 3: *Callulina meteora* in Mkingu Nature Forest Reserve

2. Aim of the study.

The focus of this study aims to understand the feeding ecology of the two similar, co-existing, and critically endangered species endemic to Mkingu Nature Forest Reserve (MNFR) amidst the ongoing rapid forest loss (Hamunyela et al. 2020); Truscott 2023) threatening their habitat. Apart from Loader et al. (2010ab) and Menegon and Gower (2011) who described the two species, no study has been dedicated to these species, and most basic ecological characteristics like their feeding, where they live, how they breed, etc., remain poorly understood. This study will determine the food composition of the two species. Considering the IUCN's 1987 policy advocating for ex-situ conservation of species at risk of extinction such as critically endangered species (IUCN 1987), understanding the dietary needs of these species becomes crucial not only for their ex-situ conservation efforts but also for enhancing in-situ conservation programs. Additionally, this study will unveil the possible impacts of the ongoing anthropogenic activities concerning their food availability in the forest and raise awareness in the adjacent communities on the importance of forest biodiversity including the amphibians of the forest particularly the two species *Callulina hanseni* and *Callulina meteora*.

3. Methodology and planned analysis

3.1 Study site and study area

This project will be conducted in Mkingu Nature Forest Reserve (MNFR), part of the Nguru Mountain block in the Eastern Arc Mountains (EAMs) ($6^{\circ}01'$ to $6^{\circ}13'S$ and $37^{\circ}26'$ to $37^{\circ}37'E$) (Figure 4). The reserve combines two former reserves, Nguru South and Mkindo Forest reserves. Mkingu Nature Forest Reserve is surrounded by 25 villages, having a combined population of 51,037 according to the 2002 census. Notably, one of these villages, Kombola, is legally enclaved within the reserve's boundaries and is home to a population of 1,382 people (TFS 2023). As of the 2022 census the population is expected to increase.

Furthermore, 29 vertebrate species endemic to the Eastern Arc Mountains are also found in the MNFR (The Arc Journal 2017). Mkingu Nature Forest Reserve encompasses 137 (25%) of the 554 plant taxa endemic to the Eastern Arc Mountains (TFS, 2023). This information signifies the conservation importance of the reserve emphasizing its pivotal role in preserving the unique biodiversity of the Eastern Arc Mountains.

MNFR is not only serving the locals with cultural values, medicinal plants, and wood sources but also holds significance as the main water catchment area supplying water to the locals in the adjacent villages and Turiani town and ultimately to the Wami River, which in turn provides water to Chalinze town.

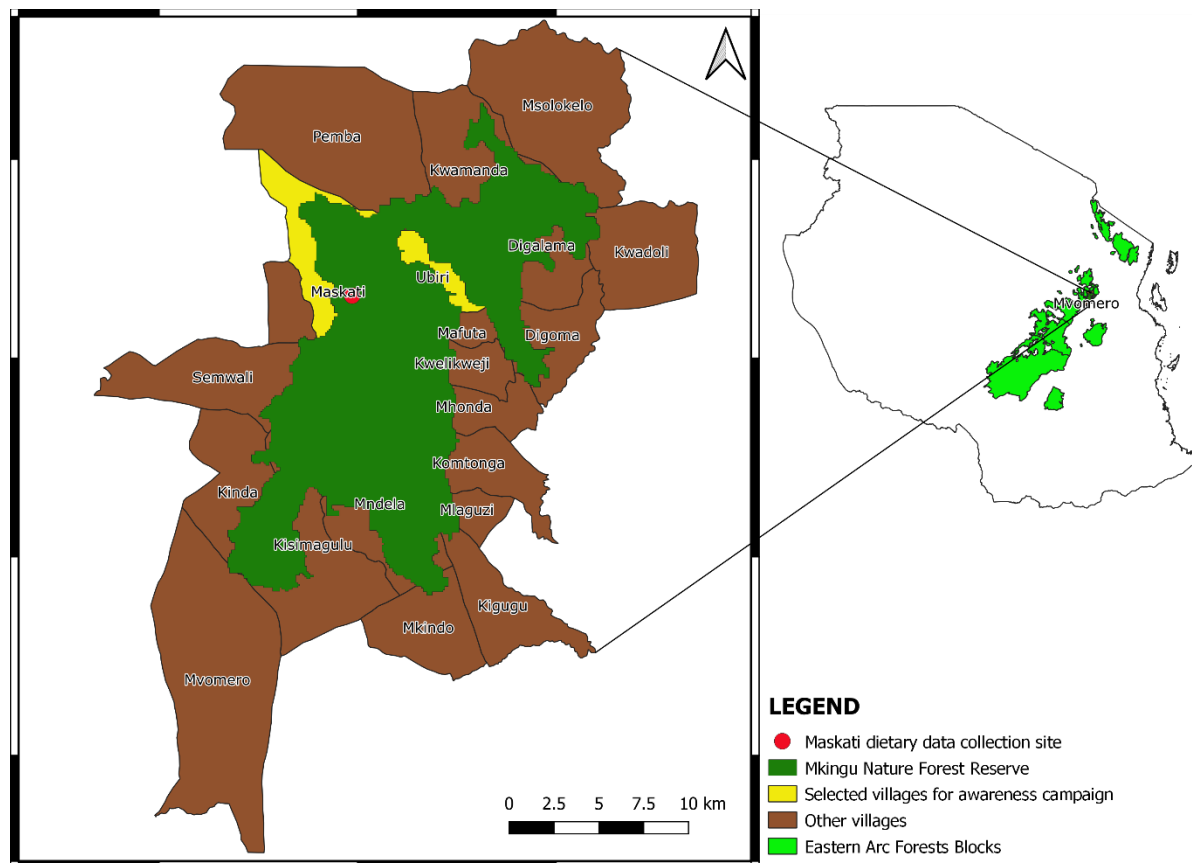


Figure 4: Map of Mkingu Nature Forest Reserve and the adjacent villages

3.2 Data collection

This project will employ the use of time constrained survey and audiovisual survey in detection of the individuals as they are the most successful methods in detection of the two species the methods were used in Menegon and Gower (2011) and are known to study *Callulina* species in other mountains (De Sa et al. 2004, Loader et al. 2009, 2010ab). Searching will be carried out at night from 2000 hours to 2200 hours, conducted by 3 individuals within 5 searching nights.

Assessing food composition: This work will employ the use of a non-invasive gut flushing method following Solé and Rödder (2010). Followed immediately after detection of individual, flushing method incorporates the use of force into a syringe connected to a pipe which is introduced to the mouth and then to the esophagus with the help of a spatula to open the mouth. The flushed contents will then be preserved in 70% ethanol for later identification to the lowest taxa possible (Marquina et al. 2021). This project will obtain food samples from individuals not less than 30 for each of the two species.

Raising community awareness: I have been conducting visits to the forest area, gaining familiarity with the villages in terms of their proximity to the forest, the extent of anthropogenic activities impacting the forest, and understanding the economic activities within these villages. I will select 2 villages depending on their closeness to the forest, the villages closer to the forest are given priority, the selected villages are Maskati and Ubiri (Figure 4). The proposed project aims to conduct detailed interviews with communities surrounding MNFR on their knowledge and perceptions regarding amphibians of the forest particularly targeted frogs, the forest, and its biodiversity in general as well as the recent anthropogenic activities and their implication to the conservation of the forest. This will involve villagers, village government officers, village environmental committees, and forest officers. Pictures of the two species and other endemics will be used during the interviews. Additionally, the proposed project will select primary and secondary schools and conduct dissemination of conservation education on the importance of the forests through presentations/seminars and discussions. The project will use the collaboration with the ongoing forest restoration efforts by the PAMS Foundation in Pemba village to enhance awareness campaigns. The restoration project is led by Andrea Biachi, a tropical botanist, with whom we have been in correspondence for more than two years.

3.3 Justification for the methods

Several methods have been used to assess feeding in amphibians. This included analyzing preserved materials, examining gut contents, gut flushing etc. (Solé and Rödder 2010, Marquina et al. 2021). Most of these methods require killing of a large number of animals, making them unsuitable especially for threatened species like the *Callulina meteora* and *Callulina hanseni*. Museum data are useful but they require large number of museum specimens, and might not reflect the current field situations; Very little museum specimens have been collected for the two target species e.g. less than ten specimens are present in vertnet database (<http://portal.vertnet.org/search>) for the two species. Stomach flushing method does not need killing the frogs, have very minimal side effects on individuals and has been proved to provide reliable data (Solé et al. 2005).

Raising awareness: Detailed interviews assisted by photos is one of the most effective methods for collecting social information and people's perceptions (Hurworth et al. 2005). Furthermore, dissemination of conservation awareness in the villages, primary, and secondary schools through pictorial presentations/seminars is both impactful and efficient, aiming to educate and reach as many people as possible within a short period.

3.4 Data analysis

The flushed contents will be identified following the available identification keys basing on their available taxonomic group. To determine the relationship between food compositions of the two species this study will use Chi-square test. Additionally, Jaccard similarity will be used to determine the similarity of the food composition between the two species.

4. Expected outcomes

Enhanced conservation strategies of the two species and their habitat: Since the IUCN calls for captive breeding of species considered at risk such as Critically Endangered species (IUCN 1987), the information that will be obtained on studying food composition of the two species will aid captive rearing of the species, if need be, in the future, and will also make it possible to critically evaluate the impact of the ongoing anthropogenic activities in the insitu conservation in relation to the frogs's food availability.

Increased community awareness on amphibians in the forest: Raising awareness on the other hand by engaging with adjacent villages and highlighting the significance of conserving these species and their habitat, this research can foster greater community involvement in conservation initiatives, ultimately contributing to the long-term protection of MNFR and its biodiversity.

5. Planned expenditure

Expenditure	Cost (€)
Travelling from Dar es salaam to Turiani Morogoro. Go and back.	74
Transport to the forest, motorcycle and potters	65
Camping cost, food	87
Flushing materials, preservatives and personal protection	169
Two assistants' allowances 5 days dietary composition data collection.	108
Entrance fee and research permit for the team	163
Bites and refreshment during discussions/ seminars.	40
Allowance for 1 - PAMS foundation forest restoration officer and 1 - MNFR officer.	72
Allowance for two villagers from village environmental committee from two villages.	36
Printing of presentation poster	35
Trasport to raise awareness in selected schools.	36
Stationeries	30
Food and shelter outside forest during awareness campaign.	85
Total	1000 €

The above expenses have been calculated based on the exchange rate of 1€ = 2,786.65 Tsh.

6. Time scale

Task	October (2024)	November	December	January (2025)	February	March
Preparation and purchasing of flushing items						
Data collection						
Awareness campaign						
Food items identification						
Data analysis						
Report writing and publication						

7. Personal skills/ experience

As an active herpetologist with over 2 years of survey experience in the Eastern Arc Mountains, particularly Uluguru and Mkingu Nature Forest Reserves, my skills in herpetofauna identification and ecological understanding have been strengthened. Acquired standards from my role as an assistant researcher guarantee the project's viability. I possess scientific writing and analytical skills for thorough data analysis. Importantly, my established network of contacts includes key stakeholders and top experts willing to support the project, emphasizing a collaborative approach essential for the success of conservation efforts in the Mkingu Nature Forest Reserve.

8. REFERENCES

- Bishop PJ, Angulo A, Lewis JP, Moore RD, Rabb GB and Moreno JG 2012 The Amphibian Extinction Crisis-what will it take to put the action into the Amphibian Conservation Action Plan? *SAPIEN. S. Surv. Perspect. Integr. Environ. Soc.* (5.2)
- Chanson, J. et al. (2008). The State of the World's Amphibians. In: Stuart et al. (Eds.) *Threatened Amphibians of the World*, pp. 33-52. Barcelona/Gland/Arlington: Lynx Edicions/IUCN/Conservation International.
- Doggart NH, Howell KM, Loader SP and Mbilinyi B 2007 The biological importance of the Eastern Arc Mountains of Tanzania and Kenya. *Biol. Conserv.* 134 (2): 209–231.
- Greenberg DA, Palen WJ, Chan KC, Jetz W and Mooers AØ 2018 Evolutionarily distinct amphibians are disproportionately lost from human-modified ecosystems. *Ecol. Lett.* 21 (10): 1530–1540.
- Hamunyela E, Brandt P, Shirima D, Do HTT, Herold M and Roman-Cuesta RM 2020 Space-time detection of deforestation, forest degradation and regeneration in montane forests of Eastern Tanzania. *Int. J. Appl. Earth Obs. Geoinf.* 88: 102063.
- Hollander FA, Titeux N and Van Dyck H 2013 Habitat-dependent prey availability and offspring provisioning explain an ecological trap in a migratory bird. *Funct. Ecol.* 27 (3): 702–709.
- Hurworth R, Clark E, Martin J and Thomsen S 2005 The use of photo-interviewing: three examples from health evaluation and research. *Eval. J. Australas.* 4 (1–2): 52–62.
- IUCN 1987 The IUCN policy statement on captive breeding
- Loader SP, Gower DJ, Müller H and Menegon M 2010a Two new species of *Callulina* (Amphibia: Anura: Brevicipitidae) from the Nguru Mountains, Tanzania. *Zootaxa* (2694): 26–42.
- Loader SP, Gower DJ, Ngalason W and Menegon M 2010b Three new species of *Callulina* (Amphibia: Anura: Brevicipitidae) highlight local endemism and conservation plight of Africa's Eastern Arc forests. *Zool. J. Linn. Soc.* 160 (3): 496–514.
- Loader SP, Measey GJ, De Sa RO and Malonza PK 2009 A new brevipitid species (Brevicipitidae: *Callulina*) from the fragmented forests of the Taita Hills, Kenya. *Zootaxa* 2123: 55.
- Lyimo E 2014 An analysis of the stakeholders and drivers of deforestation and forest degradation in the South Nguru landscape. *TFCG Tech. Pap.* 45: 1–59.
- Marquina D, Buczek M, Ronquist F and Łukasik P 2021 The effect of ethanol concentration on the morphological and molecular preservation of insects for biodiversity studies. *PeerJ* 9: e10799.
- Menegon M and Gower D 2011 *A remarkable new species of Callulina (Amphibia: Anura: Brevicipitidae) with massive, boldly coloured limb glands Snakes of India View project Conservation of caecilian amphibian of Cameroon View project.*
- Menegon M, Loader S and LYAKURWA J 2021 *Amphibians of the Tanzanian forests.*
- Nahonyo C, Goboro E, Ngalason W, Mutagwaba S, Ugomba R, Nassoro M and Nkombe E 2017 Conservation efforts of Kihansi spray toad *Nectophrynoides asperginis*: its

discovery, captive breeding, extinction in the wild and re-introduction. *Tanzania J. Sci.* 43 (1): 23–35.

De Sa RO, Loader SP and Channing A 2004 A new species of *Callulina* (Anura: Microhylidae) from the West Usambara Mountains, Tanzania. *J. Herpetol.* 38 (2): 219–224.

Solé M, Beckmann O, Pelz B, Kwet A and Engels W 2005 Stomach-flushing for diet analysis in anurans: an improved protocol evaluated in a case study in Araucaria forests, southern Brazil. *Stud. Neotrop. Fauna Environ.* 40 (1): 23–28.

Solé M and Rödder D 2010 Dietary assessments of adult amphibians. *Amphib. Ecol. Conserv. a Handb. Tech. Oxford Univ. Press. Oxford*: 167–184.

TFS. 2023. Mkingu. <https://www.tfs.go.tz/index.php/en/forests/mkingu> . Accessed on [17/03/2023]

Truscott. 2023. The value of mountains: Q&A with Tanzanian herpetologist John Lyakurwa. <https://news.mongabay.com/2023/07/the-value-of-mountains-qa-with-tanzanian-herpetologist-john-lyakurwa/>. Accessed on [15/3/2023]