Asian Journal of Research in Zoology

2(4): 1-11, 2019; Article no.AJRIZ.50166

A Preliminary Study on the Batraco-herpetological Fauna in the Forest Refuge of Albertine Rift, the Democratic Republic of the Congo

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FMMM and AL designed the study and wrote the protocol. Authors FMMM, AL and MKY carried out the field work and specimen collection. Author FMMM wrote the first draft of the manuscript. Authors FMMM and GBB managed the species identification. Authors MM, CE, PK, DA, GNB, JPKN and GT managed the literature searches and contributed to text body writing and review. Author JAA is a botanist. He had participated in the expedition and had identified the plant species in the habitats in which we had captured the amphibian specimens. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIZ/2019/v2i430076
Editor(s):
(1) Dr. Oluyomi A. Sowemimo, Senior Lecturer, Department of Zoology, Obafemi Awolowo University, Nigeria.
Reviewers:
(1) Laura Borelli Thomaz Carreira, UNESP, Brazil.
(2) Brian Crother, Southeastern Louisiana University, USA.
(3) Martin Potgieter, University of Limpopo, South Africa.
(4) Sandra Elizabeth Perez, National University of the Center of Buenos Aires, Argentina.
Complete Peer review History: http://www.sdiarticle4.com/review-history/50166

Original Research Article

Received 01 September 2019
Accepted 07 November 2019
Published 19 December 2019

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ABSTRACT

Aims: Amphibians and reptiles of the Albertine Rift in the Congolese part of the country are poorly documented. The objective of this research was to perform a preliminary inventory of the diversity of amphibians and reptiles in the region.
Study Design: This study was designed following scientific expeditions related to studies on chimpanzees. Litterature search revealed that amphibians and reptiles are poorly documented in these habitats.
Place and Duration of Study: Amphibians and reptiles were collected in 12 days between April and May 2017 in Dzu (N01.94753°; E030.88848°), Dzoo (N01.92742°; E030.89179°), Nzerku 3 (N01.94119°; E030.90612°) and Nzonzo (N01.90352°; E30.91030°).
Methodology: To collect amphibians and reptiles, we used the most minimally invasive method. This method consists of capturing 1 specimen for a known species and a maximum of 5 specimens for those for an unknown species. The surplus specimens were released into their environment. During the night between 7 pm and 9 pm, amphibians were captured by hand using a flashlight. Snakes had been captured using the snake stick. All captured specimens were scanned with a camera and then identified using amphibian and snake species identification keys. Necropsies (tongue and muscle tissue) stored in Eppendorf tubes containing alcohol (90-75%). Specimens had been fixed with formaldehyde (10%), before being preserved in alcohol (75%) in the long term. Tissues were shipped for molecular analysis to the University of Texas (United States).
Results: In the four study sites, 149 amphibian specimens were collected, consisting of 19 species, 9 genera and 8 families. According to the reptiles, 27 specimens divided into 21 species grouped into 19 genera and 11 families were recorded.
Conclusion: The batraco-herpetological fauna in the Albertine Rift in Ituri province in the Democratic Republic of the Congo is rich and diversified, hence this deserves the attention of other researchers.

Keywords: Rift Albertine; amphibians; reptiles; batraco-herpetofauna; RAFALE landscape, The Democratic Republic of the Congo.

1. INTRODUCTION

The loss of biodiversity is a major concern in the world of ecology, and its preservation is increasingly mobilizing individuals, scientists, researchers, politicians and volunteers [1]. This biodiversity is threatened by climate change and global land use, knowing that the global climate change constitutes a major threat to the survival of persistence of the biodiversity [1-5]. Environmental change directly affects the distribution and richness of species in all forest habitats [6] and the composition of assemblages [7]. The main cause of this environmental disruption and loss of biodiversity on the planet is mainly due to human activities (non-organic agriculture, deforestation, mineral exploitation, population explosion, pollution and the effects of climate change). We are called upon to understand how climate change affects biodiversity. To achieve this, we must study key organisms such as herpetofauna to better find ways to reverse the trend by proposing appropriate models for biodiversity survival.

In the east of the Democratic Republic of the Congo (DRC), the relict forests that colonize the mountainsides bordering Lake Albert in the Ituri region are rich in fauna. These forest ecosystems provide a valuable refuge for herpetofauna and other living creatures. However, these forests are considerably reduced by human activities [8]. This reduction in vegetation is detrimental to the survival of amphibians and reptiles. As a result, wetlands have become vast areas of agriculture and the rise in temperature is affecting the aquatic amphibian larvae [3].

The Albertine Rift region is very important for conservation because it contains more vertebrate species than anywhere else on the continent. A total of 295 reptile species are currently present in the Albertine Rift, of which 14.5% are endemic to the region, including two crocodile species that are endangered species but other species are listed as endangered species by IUCN. Reptiles are represented by 38 endemic species [9] while for amphibians, 45 species are endemic (25.7%), including 11 vulnerable species and 2 threatened species. It should be noted that many threats to the ecosystems of the Albertine Rift are alarming. Deforestation remains the main ecological threat in the region. A large part of the forest has been

cleared for agriculture and forestry, particularly in Rwanda and Burundi, a densely populated area (1000 inhabitants/km^2).

The RAFALE forest landscape was a forgotten and lost area where no studies were published on its highly diversified biological richness though few studies reported on its biological richness recently [10-11]. Therefore, studying the herpetological fauna of this area was an emergency. Through this study, we highlight the specific richness of amphibians and reptiles in the forest on the slopes of the Albertine Rift Mountains in the Djugu territory. The objective of this research was to perform a preliminary inventory of the diversity of amphibians and reptiles of the Albertine Rift in the Congolese part.

2. MATERIALS AND METHODS

2.1 Location of Study Sites

The four sites are located in relict forest shreds situated in Ituri in the territory of Djugu, on the shores of Lake Albert in DRC (Fig. 1). The geographical coordinates of the four sites are Dzu (N01.94753°; E030.88848°), Dzoo (N01.92742°; E030.89179°), Nzerku 3 (N01.94119°; E030.90612°) and Nzonzo (N01.90352°; E30.91030°).

2.2 Vegetation

Mountain forests are still maintained in Djugu and Mahagi territories. The northeastern territories are covered by forests of medium altitude (1200-1500 m a.s.l.) and high altitude (>1500 m a.s.l.) while those of Irumu and Mambasa are of low altitude (<1200 m). On the Lendu Plateau, vegetation is currently dominated by grassy savannah, but gallery forests persist towards Lake Albert [2]. These galleries are secondary types and border the various rivers that flow into Lake Albert. They are rich in species belonging to the families of Annonaceae, Boraginaceae, Cannabaceae, Euphorbiaceae, Fabaceae, Putrangivaceae, Sapindaceae, Sapotaceae and Rubiaceae. The underbrush is clear but the few underbrush shrubs are mainly represented by Acanthaceae and Rubiaceae (*Rothmannia* and *Coffea*). The predominant plant species are *Piper umbellatum* L. (Piperaceae), *Palisota schweinfurthii* (Commelinaceae) C.B. Clarke, *Brilantesia sp*, *Urtica sp* (Urticaceae) [10].

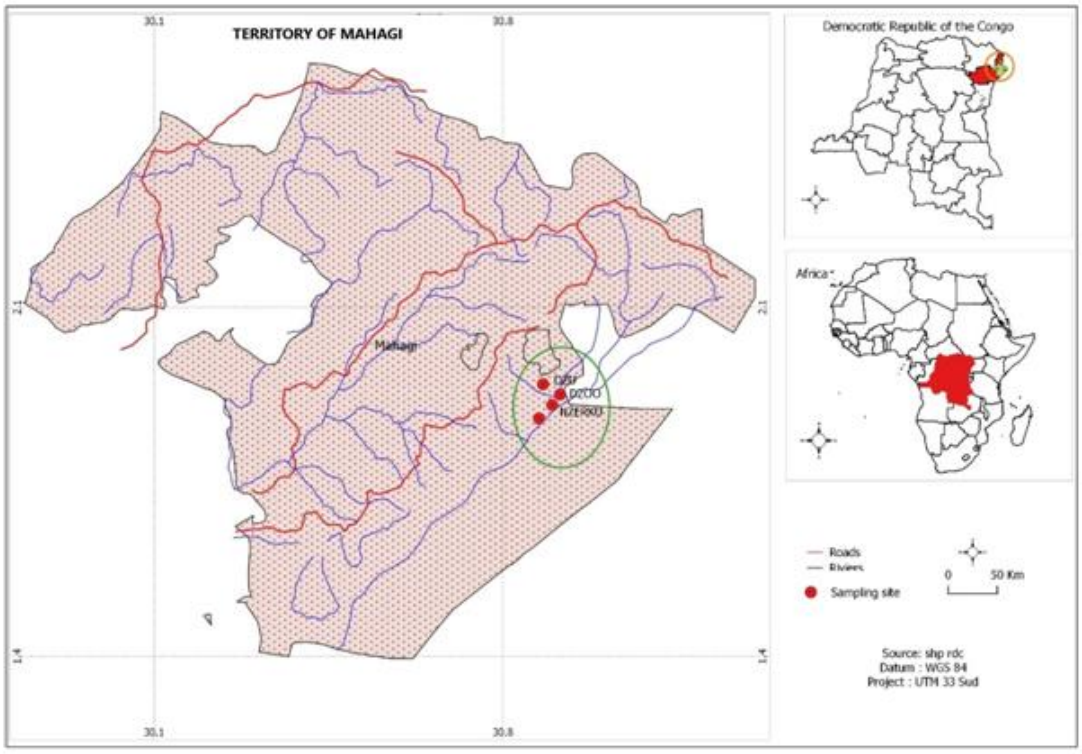

Fig. 1. Location of the four study sites in the Democratic Republic of the Congo

2.3 Methodology

2.3.1 Amphibians

For 12 days (April and May 2017), we had captured amphibians in the relict forests of Djugu. To achieve the aim of this study, the less invasive capture method was performed. This method consisted of catching one specimen for a known species and up to five specimens for those, which are difficult to identify up to the rank of the species while the other specimens were released into the forest. The amphibians were captured in two stages, early in the morning and at night.

In the morning between 6 am and 7 am, amphibians were captured by hand under the litter box and tree branches. As for night capture, amphibians were captured by hand using bright flashlight light between 7 pm and 10 pm. The captured amphibians were placed in plastic jars and then transported alive in a large plastic bag to the camp for laboratory work. The captured specimens had been digitized alive on khaki graph paper using a Nikon digital camera. Then, they were identified according to the characteristics of the external morphology in the field. In the laboratory, identification was verified using amphibian identification keys [12-14].

We had collected the necropsies, which consisted of tongue and muscle tissue and were preserved in the Eppendorf tube containing the alcohol (75%). The specimens were fixed into formaldehyde (10%), before being preserved in alcohol (70%) over the long term. Molecular analyses are being carried out at the University of Texas At El PASO (USA) for the confirmation of species using molecular techniques.

2.3.2 Reptiles

All the reptiles were captured by hand except the poisonous snakes which were captured using the herpetological cone. Reptiles were captured during the day as well as at night. The identification was verified using snake identification keys [15] and by the Reptilia Data Base site. We had digitized all the specimens captured using a Nikon brand camera and necropsies consisted of tongue and muscle tissue were collected. We had fixed the specimens with formaldehyde (10%), before being preserved in alcohol (70%) for the long term. Tissue samples were shipped for molecular analysis to the University of Texas At El PASO (USA) for species confirmation following molecular techniques.

3. RESULTS

3.1 Amphibians

The amphibian's biodiversity in the Albertin Rift in RAFALE landscape is presented in the Table 1.

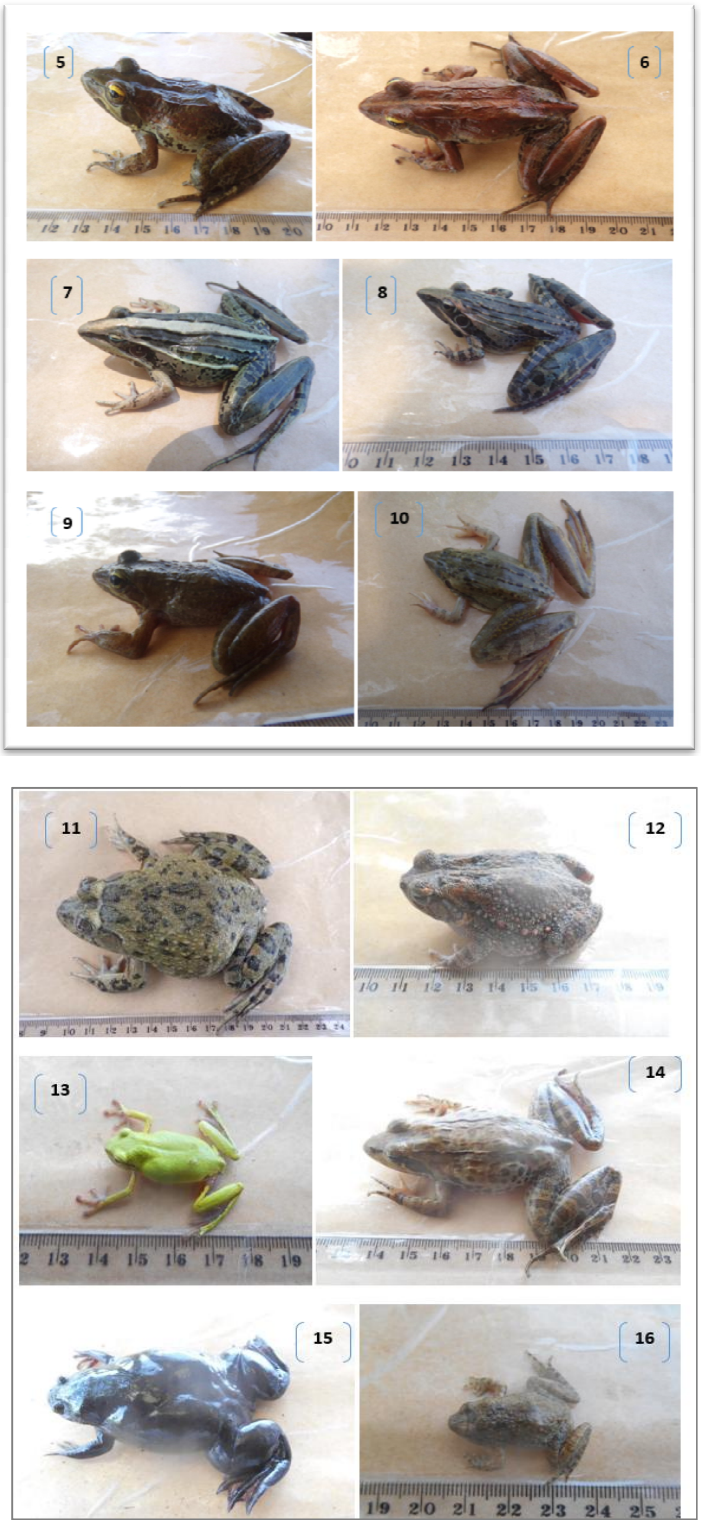

Fig. 2. Selected amphibian species captured in the RAFALE landscape

Ptychadena cf oxyrhynchus (1), Amietia sp (2, 6, 9 and 14), Amietia angolensis (5), Ptychadena sp (3, 7, 8 and 10), Hyerolius cinnamomeoventris (13), Arthroleptis sp (4 and 16), Hoplobatrachus occipitatlis (11), Sclerophrys sp (12), Xenopus sp (15), Arthroleptis sp (16)

Table 1. Amphibian's biodiversity in the RAFALE landscape

Order	Family	Genus	Species	Total	%
Anura	Hyperoliidae Laurent, 1943	Afrixalus (Laurent, 1944)	*Afrixalus fulvovittatus* (Cope, 1861)	7	4.70
	Pyxicephalidae Bonaparte, 1850	Amietia (Dubois, 1987)	*Amietia* sp.	25	16.78
			Amietia wittei (Angel, 1924)	4	2.68
			Amietia angolensis (Bocage, 1866)	12	8.05
	Arthroleptidae Mivart, 1869	Arthroleptis (Smith, 1849)	*Arthroleptis* sp.	6	4.03
	Dicroglossidae Anderson, 1871	Hoplobatrachus (Peters, 1863)	*Hoplobatrachus occipitalis* (Günther, 1858)	4	2.68
	Hyperoliidae Laurent, 1943	Hyerolius (Rapp, 1842)	*Hyerolius cinnamomeoventris* (Bocage, 1866)	2	1.34
			Hyperolius ocellatus (Günther, 1858)	3	2.01
			Hyperolius sp.	2	1.34
	Phrynobatrachidae Laurent, 1941	Phrynobatrachus (Günther, 1862)	*Phrynobatrachus* sp.	3	2.01
	Ptychadenidae Dubois, 1987	Ptychadena Boulenger, 1917	*Ptychadena cf aequiplicata* (Werner, 1898)	5	3.36
			Ptychadena cf oxyrhynchus (Smith, 1849)	3	2.01
			Ptychadena mascariensis (Duméril & Bibron, 1841)	30	20.13
	Bufonidae Gray, 1825	Sclerophrys Tschudi, 1838	*Sclerophrys cf guttularis* (Power, 1927)	3	2.01
			Sclerophrys maculatus (Hallowell, 1854)	12	8.05
			Sclerophrys regularis (Reuss, 1833)	4	2.68
			Sclerophrys sp	15	10.07
	Pipidae Gray, 1825	Xenopus Wagler, 1827	*Xenopus cf laevis* (Daudin, 1802)	6	4.03
			Xenopus sp	3	2.01
1	8	9	19	149	100

In the four study sites, 149 amphibian specimens were collected (Table 1), consisting of 19 species, 9 genera and 8 families (see sequence of species, genera and families in Table 1). Selected species are presented in Fig. 2. The family with the most abundant number of species is that of Pyxicephalidae Bonaparte, 1850 with the species Amietia sp (25 specimens: 16.78%), Amietia wittei (Angel, 1924) with (4 specimens: 2.68%), Amietia angolensis (Bocage, 1866) (12 specimens: 8.05%). The family of Ptychadenidae Dubois, 1987 then followed by 3 species Ptychadena cf aequiplicata (Werner, 1898) (5 specimens: 3.36%), Ptychadena cf oxyrhynchus (Smith, 1849) (3 specimens: 2.01%) and Ptychadena mascariensis (Dumeril & Bibron, 1841) (30 specimens: 20.13%). The Bufonidae family is the most diversified with 4 species: *Sclerophrys cf guttularis* (Power, 1927) having 3 specimens (2.01%), *Sclerophrys maculatus* (Hallowell, 1854) (12 specimens: 8.05%), *Sclerophrys regularis* (Reuss, 1833) (4 specimens: 2.68%) and *Sclerophrys sp* (15 specimens: 10.07%). The least represented is the family of Phrynobatrachidae, which is monospecific (*Phrynobatrachus sp* with 3 specimens, 2.01%).

3.2 Reptiles

Table 2 presents the reptiles captured in the Albertine Rift.

In the four study sites, 27 reptile specimens were collected as shown in the indicated table, consisting of 27 species distributed into 19 genera and 11 families (see sequence of species, genera and families in the table) as recorded in Dzoo, Dzu, Nzerku 3 and Nzonzo.

The individuals collected belong to Squamates Order. Sincidae family is the most abundant with following species: *Trachylepis quinquetaeniata* (Lichtenstein, 1823) (4 specimens: 14.81%), Pythonidae (Python sebae (Gmelin, 1789) with 3 specimens collected (11.11%). The family of Agamidae is the most diversified with 3 species but most of the genera identified are monospecific (Table 2).

4. DISCUSSION

The RAFALE landscape is a lost area of the DRC where scientific research is poorly carried out in almost all areas of life in general and amphibians in particular. This lack of studies in the field does not allow us to directly compare the findings of the current study with those of previous authors conducted in this area. However, we will compare the diversity of herpetofauna with those of studies conducted in neighbouring regions.

As for conservation, the studies of [3,12-14] recognize that the Albertine Rift is one of the most important conservation regions in Africa. It has more vertebrate species and contains many endemic species than any other region of the continent [16-20].

4.1 Amphibian Diversity

The current study on amphibians in the Dzu, Dzoo and Nzonzo portion of the Albertine Rift, and even at low elevations near Lake Albert to the Congolese side, shows that repeated and extensive amphibian research is more important because less is published on them. We are limited to make a substantive comparison of the findings of this research. We would indeed focus on the work performed by other researchers on the other part of the Rift.

The Albertine Rift is a region in Central Africa that extends 30 km in the north of Lake Albert to the southern tip of Lake Tanganyika, including the western rift valley and escarpment slopes less than 100 km east of the border with DRC. Studies on amphibians on the Albertine Rift have shown that it is particularly rich in amphibian diversity and endemism [17]. Approximately 20% of amphibians in Africa are found in this rift [18] Newer species descriptions in the genus Hyperolius [20-21] have added knowledge to the known amphibian diversity on the Albertine Rift.

Table 2. Reptile's biodiversity as listed in the RAFALE landscape

Family	Genus	Species	Total	%
Agamidae	Acanthocercus	*Acanthocercus sp*	1	3.70
	Agama	*Agama cf africana* (Lichtenstein, 1823)	1	3.70
		Agama sp.	1	3.70
Viperidae	Bitis	*Bitis arietans* (Merrem, 1820)	1	3.70
Chaemeleonidae	Chaemeleo	*Chaemeleo sp*	1	3.70
Gekkonidae	Cnemaspis	*Cnemaspis sp*	1	3.70
Colubridae	Dipsadoboa	*Dipsadoboa viridis* (Peters, 1869)	1	3.70
Lamprophidae	Duberria	*Duberria sp*	1	3.70
Gekkonidae	Hemidactylus	*Hemidactylus brookii* (Gray, 1845)	1	3.70
		Hemidactylus mabouia (Gray, de Jonnès, 1818)	1	3.70
Gekkonidae	Lygodactylus	*Lygodactylus sp*	1	3.70
	Trachylepis	*Trachylepis sp*	2	7.41
Elapidae	Naja	*Naja melanoleuca* (Hallowell, 1857)	1	3.70
Scincidae	Panaspis	*Panaspis sp*	1	3.70
Colubridae	Philothamnus	*Philothamnus angolensis* (Bocage, 1882)	1	3.70
Lamprophidae	Prosymna	*Prosymna sp*	1	3.70
	Psammophis	*Psammophis sp*	1	3.70
Pythonidae	Python	*Python sebae* (Gmelin, 1789)	3	11.11
Colubridae	Thelotornis	*Thelotornis kirtlandii* (Hallowell, 1844)	1	3.70
Scincidae	Trachylepis	*Trachylepis cf quinquetaeniata* (Lichtenstein, 1823)	4	14.81
Varanidae	Varanus	*Varanus niloticus* (Linnaeus, 1766)	1	3.70
11	19	21	27	100

Fig. 3. Selected reptile species captured in the RAFALE landscape

Cnemaspis sp (1), Trachylepis quinquetaeniata (2), Python sebae (3), Trachylepis sp (4), Philothamnus angolensis (5 and 10), Thelotornis kirtlandii (6), Duberria sp (7), Bitis arientans (8), Lygodactylus sp (9)

We have found that the batraco-herpetological fauna of the Rift is rich and diverse (Tables 1 and 2). As for the species recorded, 7 specimens of *Afrixalus fulvovittatus* were captured mainly on the Poaceae (Herbaceae) located in the swamps and most of them were vocal, especially males. Among these, we noted *Amplexus* genus while .as for the genus Amietia, they were mostly captured in open areas such as mixed maize-corn and cassava leaf fields (these fields are located near streams), while others were captured on litter in the Forest, on the rocks and on the ground. Species of this genus were generally discrete and less vocalized. From 7 pm to 9 pm, the capture activity was still active. For instance, *Arthroleptis sp* were mostly captured in the forest under a closed canopy in the morning. *Hoplobatrachus occipitalis* had not been captured except on Lake Albert. They are very active at night from 7 pm and onwards.

There were three species of the *Hyperolius* genus which were captured in swampy areas dominated by Poaceae where they were actively vocalized during the night. *Phrynobatrachus sp* was captured during the night in the shreds of forests on a small branch of tree located not far from the ground. Most Ptychadena species were captured on the edge of rivers from 7 pm onwards. All the inventoried Sclerophrys species were found on litter in the forest and others were completely in the water. We captured *Xenopus* genus in muddy marshes containing water and rotten foliage with twigs of trees.

4.2 Reptilian Diversity

The Ituri forest and the Rift are deemed to be more diversified in the herpetofauna. Many species have already been described in the region of Mungbwalu, such as *Kinyongia gyrolepis sp. nov*. [22]. In total, 27 reptile specimens were captured and these specimens were distributed into 21 species, 19 genera and 11 families. The majority of these reptiles were captured during the day, mainly specimens belonging to the Agamidae family except *Cnemaspis sp* which was captured on litter in a hollow tree after the rain. Meanwhile, *Bitis arietans* was captured at dusk in a tuft of dry grass in a rocky environment and *Dipsadoboa viridis* was captured at night at the bottom of a rocky cliff with a stream flowing down it. On the other hand, *Thelotornis kitlandii* who had swallowed a *Chaemeleo sp* had been caught during the day. This snake was perched on a tree branch.

5. CONCLUSION

The aim of this study was to assess the biodiversity of batraco-herpetological fauna in the Albertin Rift, Ituri province in DRC precisely in the RAFALE landscape. The findings show that RAFALE's relict forests are rich in amphibians and reptiles. However, in the future, it would be important to verify the phylogeny of certain species in different forest blocks of this region which are separated by ecological barriers. To achieve this, more elaborate ecological studies must be undertaken to highlight the ecological role, the diversity between different sites and possibly specifically compare the abundance, constancy, or even rarity of batraco-herpetological fauna from one site to another.

Finally, this study shows that it is possible to carry out research in the Djugu area, although it is considered a "red zone" and it has made it possible to advocate with the DRC authorities to strengthen laws and promote this site of significant biological value so that it benefits from a special protection status at the national, or even international level, for the conservation of its rich and varied biodiversity resources.

ETHICAL APPROVAL

As per the international standard, a written ethical permission has been collected and preserved by the author(s).

ACKNOWLEDGEMENTS

We thank Dr Eli Greenbaum (University of Texas at El Paso, USA) for personally funding the herpetological part of the expedition. We also want to thank Caroline Thrion (Director), Sam (Cameraman, and Jean Michel (Deputy Director) of the GEDEON Program for filming these mountain regions and making it visible around the world through the FEST FIL EURO program. We thank the Biodiversity Monitoring Centre of the University of Kisangani for the logistics and scientific staff. May our field guide DRAMANI find here his merits just to his value of having braved this rugged terrain at night as well as during the day.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/50166