



Article

# Diversity and Endemism of Amphibian Fauna in the Yoko Forest Reserve, Democratic Republic of the Congo

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Abstract: This article provides the first data on amphibian diversity in the Yoko Forest Reserve, located in the Democratic Republic of the Congo. During twenty-four field sampling campaigns organized over a period of twelve months, amphibians were collected from nocturnal surveys supported by three techniques: visual spotting using a headlamp, systematic searching of habitats and acoustic hearing of vocalizations. A total of 5707 amphibians in 10 families, 17 genera and 33 species were recorded throughout the study area. The Hyperoliidae and Arthroleptidae families were the most diverse, with *Amnirana albolabris* (Ranidae) the most abundant species, followed by *Phrynobatrachus auritus* (Phrynobatrachidae). By contrast, *Afrixalus quadrivittatus*, *A. equatorialis*, *Arthroleptis tuberosus*, *A. variabilis*, *Cryptothylax greshoffi*, *Hyperolius langi*, *H. ocellatus*, *H. parallelus*, *Hyperolius* sp., *Hoplobatrachus occipitalis*, *Kassina maculosa*, *Leptopelis calcaratus*, *Nectophryne batesii*, *Phrynobatrachus perpalmatus*, *Sclerophrys gracilipes* and *S. gutturalis* were less frequent. For the first time, *Amietia nutti* and *Kassina maculosa* have been reported in Congolese forests. Amphibian species known from the YFRE are widely distributed in Central African forests, and particularly in the Democratic Republic of the Congo, where *A. equatorialis*, *H. langi*, *H. parallelus* and *Ptychadena christyi* are endemic.

Keywords: amphibians; Yoko forest reserve; endemism



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# 1. Introduction

Amphibians are a widely distributed and diverse taxon, with 8742 known species [1], of which 969 and 336 species have been inventoried in the Afro-tropical region [2] and in Central Africa, respectively [3,4]. Congolese forests are home to 254 amphibian species, of which 59 are endemic [1].

Despite their great diversity, knowledge of amphibians remains limited in many parts of the world [5–8]. However, wherever they occur, they are disappearing at an alarming rate, mainly as a result of human activities [9,10]. As a biodiversity hotspot,

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the Democratic Republic of the Congo (DRC) is particularly concerned by this situation. The literature on amphibians reveals a number of studies that have been carried out in the DRC. Some of these have described a few genera [11,12], while others have focused on specific compositions [13–16]. This disappearance of amphibians also concerns the forest areas surrounding the city of Kisangani, which have been altered by slash-and-burn agriculture and timber exploitation, hence threatening biodiversity, which remains insufficiently understood. It is in this context that the present study was carried out in the Yoko Forest Reserve (YFRE) in order to document the presence and diversity of amphibians.

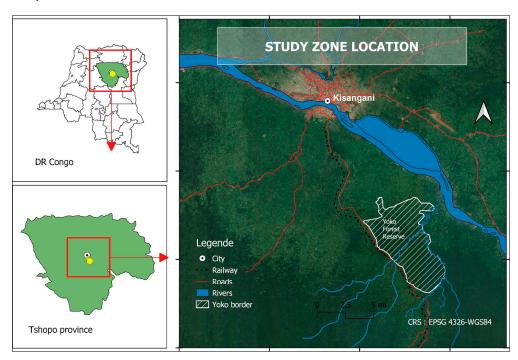
Although amphibian diversity in the YFRE has not yet been entirely described, the available literature [17,18] suggests that the amphibian fauna in this forest ecosystem is rather diverse. Of all known amphibian species, Amnirana albolabris and A. galamensis are the most abundant, due to their wide distribution in sub-Saharan Africa and their ability to cross geographical barriers and forest refuges [19]. These species could also be the most abundant in the YFRE, located in the vicinity of the town of Kisangani, an area intersecting the East-Central and South-Central faunal regions of the Congo Basin, which are reputed to be very rich in animal species [20,21]. It is therefore assumed that the Reserve would host a higher species richness than other Central African forests. Given that the fauna expected in the various Central African forest blocks are different, there should be little similarity between the YFRE and the other Central African forests. On the other hand, a high degree of similarity is expected between the YFRE and comparable sites in the DRC, due to a large number of common species. Considering the research carried out in Africa [22,23], which limits the distribution of certain species to Central Africa and particularly to the DRC, we can expect endemism rates in the YFRE similar to those found in other Central African forests.

This study aimed to determine the diversity of amphibians in the YFRE. The objectives were to provide initial information on the specific composition and richness of amphibians in order to create a checklist for the study area, and to compare this with data from reference studies carried out in other forest blocks in the DRC and Central Africa as a whole. The YFRE is one of the forest ecosystems located around the city of Kisangani, and the amphibian species it harbors provide a motivation for understanding the ecological roles of forests in the Kisangani region.

## 2. Materials and Methods

The YFRE is located near the equator, between 0°15′ and 0°20′ N and 25°14′ and 25°20′ E [24], on the road connecting Kisangani with Ubundu, in the Tshopo Province (Figure 1). It is characterized by an average annual temperature of 25 °C, an average annual rainfall of 1750 mm and by the absence of dry months [25]. As throughout the Kisangani region, rainfall throughout the year is interrupted by two sub-dry seasons. The long rainy season runs from September to December and the short rainy season covers the months from March to June. On the other hand, the long sub-dry season extends through January and February and the short sub-dry season covers July and August [26,27]. Vegetation is dominated by original forest flora interspersed with fallow land and fields resulting from human activity. Thus, from one place to another there are pockets of primary forest dominated by Manniophyton fulvum Mull. Arg (Euphorbiaceae), Trilepisium madagascariense Dc. (Moraceae), Vitex congolensis De Wild. & Th. Dur. (Lamiaceae), Pterocarpus soyauxii Taub. (Fabaceae), Zanthoxylum gilletii (De Wild.) P.G. Waterman (Rutaceae), Chrysophyllum pruniforme Pierre ex Engler. (Sapotaceae), Anonidium mannii (Oliver) (Engler & Diels) (Annonaceae), Microdesmis yafungana J. Léonard (Pandaceae), Scaphopetalum thonneri De Wild. & Th. Dur. (Malvaceae), Diospyros melocarca F. White (Ebenaceae), Heisteria parviflora Smith. (Olacaceae), Megaphrynium macrostachyum (Bentham) Milne-Redh and Marantochloa mannii (Bentham) Milne-Redh (Maranthaceae). The hydrographic network is dominated by rivers, the most important of which are the Yoko and Biaro streams. They receive water from numerous small streams and rivers before discharging into the Congo River. The Diversity **2024**, 16, 457 3 of 17

Yoko River, which gave the studied reserve its name, flows from west to east, dividing the study zone into two blocks, north and south.



**Figure 1.** Location map of the Yoko Forest Reserve in the Kisangani region, Tshopo Province, Democratic Republic of the Congo. Data sources: background (https://mt1.google.com/vt/lyrs=s&  $x=\{x\}$ & $y=\{y\}$ & $z=\{z\}$ , accessed on 15 February 2023; city, railway, roads, rivers and Yoko border layers (RGC: https://www.rgc.cd, accessed on 15 February 2023).

Data were collected in twenty-four field campaigns, situated over a twelve-month period from December 2020 to November 2021, with two six-day campaigns per month. The method used for data collection was nocturnal capture. It was supported by three techniques, including visual survey, systematic excavation of habitats using a headlamp and acoustic survey by hearing vocalizations. According to [28], the first two techniques are less sensitive to individual detection and less subject to gender bias, while the third technique favors male detectability. Specimens were collected manually over a period of three consecutive months and, once identification had been mastered, only doubtful specimens or those belonging to species not yet recorded were collected. After having removed the tissues needed for genetical analysis, unknown specimens were euthanized, labeled, fixed with 4% formalin and preserved in 70% alcohol. They were identified at the Centre de Surveillance de la Biodiversité and deposited at the Laboratoire d'Ecologie et Gestion des Ressources animales at the University of Kisangani, located in the city of Kisangani (0°30′34.3″ N, 25°11′31.2″ E), DRC. The remaining specimens were regularly released after a simple, standard pre-marking procedure involving permanent dyes applied to the head or back of the individuals, in accordance with the capture-mark-recapture method [29,30]. From the seventh session onwards, individuals belonging to known species were photographed in situ. They were then identified based upon the descriptions in [23,31–37] before being released. During collection, data were transcribed onto specially designed cards and then digitized for further analyses.

To determine the diversity of amphibians in the study area, Simpson's index ( $D_s$  or 1-D) was calculated from Equation (1). This is one of the most significant and robust measures of diversity, summarizing the state of biodiversity in the study area [17,18].

$$D_S = 1 - \sum_{i=1}^{S} (p_i)^2 \tag{1}$$

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where  $p_i$  is the proportion of individuals of species i ( $p_i = n_i/N$ , where  $n_i$  is the number of individuals of species i and N is the total number of individuals collected). The values obtained can vary between 0 (only one species present) and ~1 (same abundance for all species); they provide information on the probability that two individuals taken at random from a given stand belong to two different species. In this way, we expect to obtain a value close to 1 (hypothesis 1) because the abundance is expected to be the same for all species present in the study area, and since the surveyed zone is assumed to be very diverse. The rarefaction curve [38–40] was generated to assess the effectiveness of the sampling based on the species richness obtained. It shows whether the study has counted all the species present in the environment, or whether there are certain species that may have escaped sampling. According to [41], its growth is expected at the start of the captures, followed by its flattening out because the sampling effort is sufficient, indicating that the number of species found is representative of the study area.

To test whether all species are equally abundant or not (hypothesis 2), we calculated species abundances as a percentage of each species in the total catch of individuals. To compare the species richness of the YFRE with the other study areas, a rank distribution of species richness was made so that it could be used to rank the sites compared according to species richness. We compared species richness among several areas to see where the YFRE ranked. We expected the YFRE, due to its size and location, to rank at an intermediate position among several areas compared (hypothesis 3). To detect similarities between this study and others that have been carried out in the DRC and Central Africa, Jaccard's dissimilarity was calculated using formula (2) [42]. We expected to find the areas concerned grouped in more than one group based on geography and species richness (hypothesis 4). This is an asymmetrical binary coefficient [42,43] chosen because quantitative data could not be collected from the comparative studies; as only species lists were available, comparisons could only be made based on presence—absence data. The asymmetry of Jaccard's index is important since the comparison of double absences would be meaningless in this study.

$$S_7 = \frac{a}{(a+b+c)} \tag{2}$$

where  $S_7$  represents Jaccard's similarity; a represents the number of species present in two sites; and b and c are the number of species present in one site but not in the other. Thus, dissimilarity was calculated from relationship (3). The need to use this dissimilarity comes from the fact that in the analyses we used the "vegan 2.6.4" software package and the "vegdist" function, with the latter always outputting the dissimilarity [42,43].

$$D = 1 - S_7 \tag{3}$$

To calculate similarities between the studies compared in this article, all unidentified species were removed from the analyses. However, unidentified species were considered to establish the ranks of specific richness, as well as in the calculation of endemism rates. The choice of the UPGMA (unweighted pair group method with arithmetic mean) grouping method was determined by the value of the cophenetic correlation coefficient. Indeed, the highest value of the cophenetic correlation coefficient indicates the grouping method that best represents the information present in the dissimilarity matrix [42,43]. Endemism rates were obtained by dividing the number of endemic species found in a study by the total number of species recorded in the same study. We expected the YFRE to score higher than other areas tested regarding the rate of endemism (hypothesis 5). For this, we calculated and compared the endemism percentage in each site. The endemicity of species in Central Africa and the DRC was determined using data provided by [22,23]. Field data were processed in Excel 2016. Past 4.13 software was used to generate the rarefaction curve. All subsequent analyses were carried out using R.4.3.1 software (through RStudio 2023.12.1+402) [44]. Simpson's diversity index was calculated using the "BiodiversityR 2.15-3" package. Dissimilarities between studies were calculated using the "Vegan 2.6-4" Diversity 2024, 16, 457 5 of 17

package and the "vegdist" function [45]. However, the dendrogram illustrating dissimilarities between the studies compared was produced using the "factoextra 1.0.7" package [46]. The graph illustrating specific richness ranks was adapted from that on abundance ranks proposed by [47], using the "BiodiverstyR" package. The location maps for the YFRE and the different study areas in Central Africa were produced using, respectively, QGIS 3.16.3 and QGIS 2.18.18.

#### 3. Results

A total of 33 species in 17 genera and 10 families belonging to the Anura order were recorded in the YFRE (Table 1). All species present in the study area could have been documented by this study (Figure 2). The most species-rich families were Hyperoliidae (n = 11; 33.33%) and Arthroleptidae (n = 8; 24.24%), followed by Bufonidae (n = 4; 12.12%), Ptychadenidae (n = 3; 9.09%) and Phrynobatrachidae (n = 2; 6.06%). All other families were monospecific (3.03%). These were the Ranidae, Dicroglossidae, Pipidae, Pyxicephalidae and Rhacophoridae. Figure 3 shows the 33 species present in the YFRE. All species are in the "Least Concern" category (LC), except for *Arthroleptis tuberosus* which belongs to the "Data Deficient" category (DD) according to the IUCN Red List categorizations. For the first time, species *Amietia nutti* and *Kassina maculosa* were recorded in the lowland forests around the town of Kisangani. Simpson's index gave a value of 0.841, indicating that amphibians within the study area are diverse. These data on the amphibian fauna in the YFRE confirm the first hypothesis of this study.

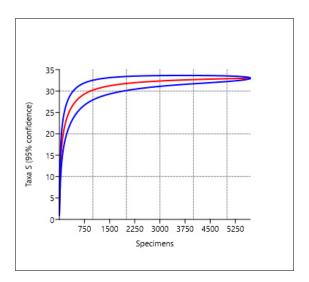
**Table 1.** Amphibian species recorded between December 2020 and November 2021 in the Yoko Forest Reserve (Democratic Republic of the Congo) using three techniques: visual survey (VS), systematic excavation of habitats (SE) and acoustic survey (AS).

Family	Species	Techniques
	Arthroleptis variabilis Matschie, 1893	VS, SE
	Arthroleptis tuberosus Anderson, 1905	VS, SE
	Leptopelis calcaratus Boulenger, 1906	VS, AS
Arthroleptidae Mivart, 1869	Leptopelis ocellatus Mocquard, 1902	VS, AS
Artifoleptidae Mivart, 1869	L. notatus Buchloz & Peters in Peters, 1875	VS, AS
	Leptopelis christyi Boulenger, 1912	VS, AS
	Leptopelis millsoni Boulenger, 1895	VS, AS
	Cardioglossa leucomystax Boulenger, 1903	VS
	Sclerophrys gutturalis Power, 1927	VS, AS
Pulanidas Cray 1925	Sclerophrys pusilla Mertens, 1937	VS, AS
Bufonidae Gray, 1825	Sclerophrys gracilipes Boulenger, 1899	VS, AS
	Nectophryne batesii Boulenger, 1913	VS
Dicroglossidae Anderson, 1871	Hoplobatrachus occipitalis Günther, 1858	VS, AS
	Hylambates verrucosus Boulenger, 1912	VS, AS
	Afrixalus quadrivittatus Werner, 1908	VS, AS
	Afrixalus osorioi Ferreira, 1906	VS, AS
	Afrixalus equatorialis Laurent, 1941	VS, AS
	Hyperolius ocellatus Günther, 1858	VS, AS
Hyperoliidae Laurent, 1943	Hyperolius platyceps Boulenger, 1900	VS, AS
	Hyperolius langi Noble, 1924	VS, AS
	Hyperolius parallelus Günther, 1858	VS, AS
	Hyperolius sp.	VS, AS
	Cryptothylax greshoffi Schilthuis, 1889	VS
	Kassina maculosa Sternfeld, 1917	VS
Phrynobatrachidae Laurent, 1941	Phrynobatrachus auritus Boulenger, 1900	VS
THYHODatrachidae Laufent, 1941	P. perpalmatus Boulenger, 1898	VS
Pipidae Gray, 1825	Xenopus pygmaeus Loumont, 1986	VS, SE

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 Table 1. Cont.

Family	Species	Techniques		
	Ptychadena christyi Boulenger, 1919	VS, SE, AS		
Ptychadenidae Dubois, 1987	Ptychadena perreti Guibe & Lamotte, 1958	VS, SE, AS		
	P. mascareniensis Duméril & Bibron, 1841	VS, SE, AS		
Pyxicephalidae Bonaparte, 1850	Amietia nutti Boulenger, 1896	VS, SE, AS		
Ranidae Rafinesque, 1814	Amnirana albolabris Hallowel, 1856	VS, AS		
Rhacophoridae Hoffman, 1932	Chiromantis rufescens Günther, 1869	VS, AS		
10 families	33 species			



**Figure 2.** Species rarefaction curve generated from amphibian sampling in the Yoko Forest Reserve. Rapid growth was observed at the start of the captures across the abundant species. Subsequently, the curve stabilized as captures evolved, because only rare species were recorded. The abscissa represents the number of individuals and the ordinate represents the number of species. The red line indicates the data collected and the blue lines represent the 95% confidence interval.



Figure 3. Cont.

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Figure 3. Cont.

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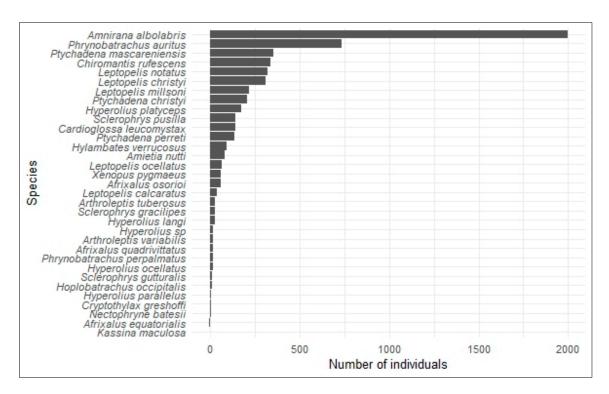
Figure 3. Amphibian species recorded in the Yoko Forest Reserve. Arthroleptidae family: *Arthroleptis variabilis* (1), *A. tuberosus* (2), *Leptopelis calcaratus* (3), *L. ocellatus* (4), *L. notatus* (5), *L. christyi* (6), *L. millsoni* (7), *Cardioglossa leucomystax* (8); Bufonidae family: *Sclerophrys gutturalis* (9), *S. pusilla* (10), *S. gracilipes* (11), *Nectophryne batesii* (12); Dicroglossidae family: *Hoplobatrachus occipitalis* (13); Hyperoliidae family: *Hylambates verrucosus* (14), *Afrixalus quadrivittatus* (15), *A. osorioi* (16), *A. equatorialis* (17), *Hyperolius ocellatus* (18), *H. platyceps* (19), *H. langi* (20), *H. parallelus* (21), *Hyperolius* sp. (22), *Cryptothylax greshoffi* (23), *Kassina maculosa* (24); Phrynobatrachidae family: *Phrynobatrachus auritus* (25), *P. perpalmatus* (26); Pipidae family: *Xenopus pygmaeus* (27); Ptychadenidae family: *Ptychadena christyi* (28), *P. perreti* (29), *P. mascareniensis* (30); Pyxicephalidae family: *Amietia nutti* (31); Ranidae family: *Amnirana albolabris* (32); Rhacophoridae family: *Chiromantis rufescens* (33) (photographed by L. Musubaho (1–8,13–25,27,31–33) and G. Badjedjea (9–12,26,28–30)).

The species rarefaction curve based on the number of individuals collected (Figure 2) shows that sampling was well representative of the expected amphibian richness for this reserve, because the data obtained reached the asymptote of the curve. A substantial decrease in slope was observed as the number of individuals increased. The most abundant species were found at the beginning, after which only rare species were recorded. The curve shows that all species present in the YFRE were observed and their number may not exceed thirty-five despite the additional sampling effort.

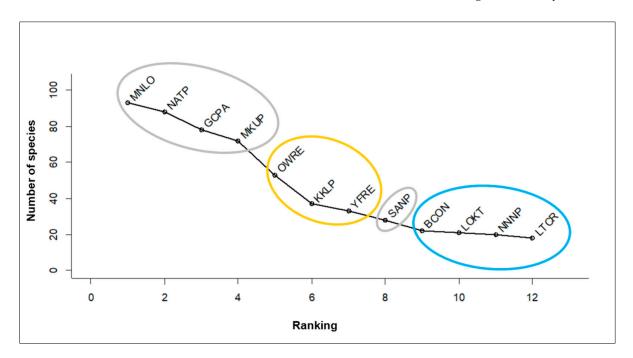
A total of 5 707 individuals were collected in the YFRE (Figure 4). *Amnirana albolabris* was the most abundant species (n = 1995), followed by *Phrynobatrachus auritus* (n = 734). In contrast, *Leptopelis calcaratus*, *Arthroleptis tuberosus*, *A. variabilis*, *Sclerophrys gracilipes*, *S. gutturalis*, *Hyperolius* sp., *H. langi*, *H. ocellatus*, *H. parallelus*, *Afrixalus quadrivittatus*, *A. equatorialis*, *Phrynobatrachus perpalmatus*, *Hoplobatrachus occipitalis*, *Cryptothylax greshoffi*, *Nectophryne batesii* and *Kassina maculosa* were the least abundant species in the study area (n < 50). The intermediate abundances were those of *Chiromantis rufescens*, *Amietia nutti*, *Ptychadena perreti*, *P. mascareniensis*, *P. christyi*, *Xenopus pygmaeus*, *Hyperolius platyceps*, *Hylambates verrucosus*, *Afrixalus osorioi*, *Sclerophrys pusilla*, *Leptopelis ocellatus*, *L. notatus*, *L. millsoni*, *L. christyi* and *Cardioglossa leucomystax*. The high abundance of the species *Amnirana albolabris* partially confirms the second hypothesis of this study because *Amnirana galamensis* is lacking in the YFRE.

The number of species varies between YFRE and studies carried out in different zones in Central Africa (Figure 5). In descending order, the species richness was as follows: Mount Nlonako (n = 93), National Parks (n = 88), Gamba Complex of Protected Areas (n = 78), Mount Kupe (n = 72), Okapi Wildlife Reserve (n = 53), Kokolopori (n = 37), Yoko Forest Reserve (n = 33), Sites Adjacent to three National Parks (n = 28), Bas-Congo (n = 22), Lokutu (n = 21), Nouabale-Ndoki National Park (n = 20) and Lake Télé Community Reserve (n = 18).

This variation in species richness between the different studies may be linked to the size and rate of disturbance of the sites, associated with the methods, the techniques used and the sampling effort engaged. The fact that the YFRE is characterized by an intermediate species richness compared to other areas enables a partial confirmation of the third hypothesis of this study. Nevertheless, Figure 5 shows a grouping of areas with similar specific richness and geographical locations, as shown in Figure 6.

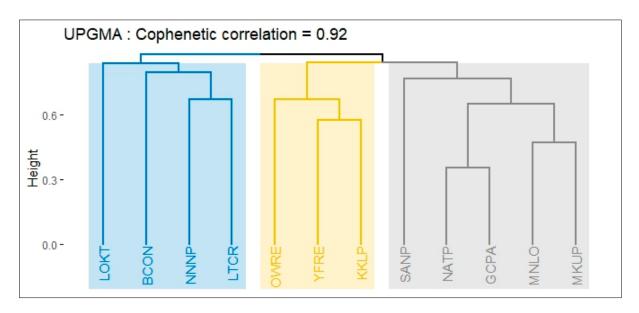


**Figure 4.** Amphibian species abundance in the Yoko Forest Reserve. *Amnirana albolabris* alone accounts for over a third of the total number of individuals caught in the study area.



**Figure 5.** Classification of studies carried out in four Central African countries based on their specific richness. BCON: Bas-Congo [14], KKLP: Kokolopori [16], LOKT: Lokutu [13], OWRE: Okapi Wildlife Reserve [48], YFRE: Yoko Forest Reserve (our study) in the Democratic Republic of the Congo; MNLO: Mount Nlonako [49], MKUP: Mount Kupe [50] in Cameroon; NNNP: Nouabale-Ndoki National Park [51], LTCR: Lake Télé Community Reserve [52] in Congo; GCPA: Gamba Complex of Protected Areas [53], NATP: National Parks [54], SANP: Sites Adjacent to three National Parks [55] in Gabon. On the abscissa are the study classes established according to the number of species on the ordinate.

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**Figure 6.** Jaccard's dissimilarity dendrogram between studies carried out in four Central African countries, based on their specific compositions. BCON: Bas-Congo [14], KKLP: Kokolopori [16], LOKT: Lokutu [13], OWRE: Okapi Wildlife Reserve [48], YFRE: Yoko Forest Reserve (our study) in the Democratic Republic of the Congo; MNLO: Mount Nlonako [49], MKUP: Mount Kupe [50] in Cameroon; NNNP: Nouabale-Ndoki National Park [51], LTCR: Lake Télé Community Reserve [52] in Congo; GCPA: Gamba Complex of Protected Areas [53], NATP: National Parks [54], SANP: Sites Adjacent to three National Parks [55] in Gabon.

In terms of specific composition, the YFRE shows the lowest dissimilarity (0.575) with the Kokolopori forest. The Gamba complex of protected areas in Gabon shows a certain similarity with all other studies carried out in Gabon, and most notably the one carried out in all protected areas in Gabon, with a similarity of 0.6483 (i.e., a dissimilarity of 3.3517). The Kupe and Nlonako mountains in Cameroon show a dissimilarity of 0.4712, i.e., a similarity of 0.5288 (Figure 6). The data provided allow us to group together our study zone, the Kokolopori forest and the Okapi Wildlife Reserve, all located in the DRC. On the other hand, Lokutu and Bas-Congo in the DRC are grouped with the Nouabale-Ndoki National Park and the Lac Télé Community Reserve in the Congo. Cameroon and Gabon are grouped together because their faunal compositions are similar, an observation based on studies carried out in both countries. These observations partially confirm the fourth hypothesis formulated in this study.

Of a total of 33 species recorded in the Yoko RF, six are endemic to Central Africa. These are *Arthroleptis tuberosus*, *Kassina maculosa*, *Leptopelis ocellatus*, *Nectophryne batesii*, *Ptychadena perreti* and *Xenopus pygmaeus*. Four species (*Afrixalus equatorialis*, *Hyperolius langi*, *H. parallelus* and *Ptychadena christyi*) are endemic to the DRC. The rate of endemism in Central Africa varies according to the number of species recorded by the various studies (Table 2). In decreasing order, the rate is 28.6% in sites adjacent to national parks and 23.9% in all national parks in Gabon; 23.8% at Lokutu in the DRC; 20.4% at Mount Nlonako in Cameroon; 18.2% in the YFRE in the DRC; 18.1% at Mount Kupe in Cameroon; 16.7% in the Lac Télé Community Reserve in Congo and the Gamba Protected Area Complex in Gabon; 16.2% in the Kokolopori Forest in DRC; 15.0% in the Nouabale-Ndoki National Park in Congo; 13.6% in Bas-Congo; and 9.4% in the Okapis Wildlife Reserve in DRC. These differences may be linked to habitat conditions, with which we associate the influence of ecological barriers on species distribution.

According to the available literature, there is no pre-existing categorization of the rate of endemism in Central Africa. Thus, based on the endemism ranking of the YFRE (6 endemic species out of 33 species recorded, i.e., 18.2%), we consider that this reserve, being the fifth among so many other surveyed zones, has a rather high rate of endemism. In

terms of endemism in the DRC (Table 3), a rate of 13.5% was recorded in Kokolopori; 12.1% in the YFRE; 11.3% in the Okapi Wildlife Reserve; 4.8% in Lokutu; and 4.5% in Bas-Congo. The fifth hypothesis of this study is hence confirmed by these results, which show that the YFRE is characterized by an appreciable rate of endemism in the DRC and Central Africa.

**Table 2.** Amphibian endemism rate in Central Africa based on studies carried out in four countries (Democratic Republic of the Congo, Cameroon, Republic of the Congo and Gabon). LS: listed species number; ESCA: endemic species number in Central Africa; %End: endemism rate.

Country DRC				Cameroon		Congo		Gabon				
Location	KKLP	BCON	LOKT	OWRE	YFRE	MNLO	MKUP	NNNP	LTCR	SANP	NATP	GCPA
LS	37	22	21	53	33	93	72	20	18	28	88	78
ESCA	6	3	5	5	6	19	13	3	3	8	21	13
%End	16.2	13.6	23.8	9.4	18.2	20.4	18.1	15.0	16.7	28.6	23.9	16.7

Table 3. Amphibian endemism rate in the DRC, assessed through studies carried out in different zones in the DRC (YFRE, OWRE, LOKT, BCON and KKLP). ESDRC: endemic species in the DRC. BCON: Bas-Congo [14], KKLP: Kokolopori [16], LOKT: Lokutu [13], OWRE: Okapi Wildlife Reserve [48], YFRE: Yoko Forest Reserve (our study) in the Democratic Republic of the Congo; MNLO: Mount Nlonako [49], MKUP: Mount Kupe [50] in Cameroon; NNNP: Nouabale-Ndoki National Park [51], LTCR: Lake Télé Community Reserve [52] in Congo; GCPA: Gamba Complex of Protected Areas [53], NATP: National Parks [54], SANP: Sites Adjacent to three National Parks [55] in Gabon.

Country			DRC		
Location	YFRE	OWRE	LOKT	BCON	KKLP
LS	33	53	21	22	37
<b>ESDRC</b>	4	6	1	1	5
%End	12.1	11.3	4.8	4.5	13.5

### 4. Discussion

Amphibian survey methods vary rather significantly [56,57]. The use of nocturnal capture supported by visual tracking, acoustic hearing and systematic habitat excavation is justified by its cost-effectiveness in the study by Anurans [41,58,59]. High humidity, moderate temperature and protection from predators [60–62] are factors that explain the intensity of nocturnal activity in amphibians, as that of their predators gradually slows down with nightfall. The individuals captured were marked and then released, because conservation is an issue that requires the maintenance and preservation of ecosystem health.

The rarefaction curve showed an increase in the number of common species at the start of sampling, which then stabilized as rare species were recorded. According to [40], rarefaction curves offer an advantage in assessing the specific diversity of a region, as they enable the number of species recorded to be linked to the sampling effort involved. Like in [63], the high diversity of the amphibian fauna in the YFRE may be explained by the availability and abundance of food resources in the various habitats that are specific to each species. Beyond these factors, [64] indicate that this diversity could be explained by the resilience of the habitats regarding anthropogenic disturbances. In the YFRE, the Hyperoliidae and Arthroleptidae families are the most diverse, while the Ranidae family is richer in individuals than all the other families. These results are confirmed by other studies carried out in the DRC [13,14,16]. Hyperoliidae and Ranidae are known to be widely distributed in Central Africa [23,36,65], where they have produced significant adaptive radiation [22]. The Hyperoliidae family is known throughout sub-Saharan Africa [34].

Of the 455 species of Ranidae known worldwide [1], only *Amnirana albolabris* occurs in the YFRE. Its high abundance may be linked to the availability of food and its strategy to escape from its predators (the species can hide itself in high places in shrubs and

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trees) [66–68]. The habitats of this species and its confinement are recognized in the humid forests [69,70] to which the forests of the DRC belong. According to [19], frogs of the genus Amnirana are widely distributed in sub-Saharan Africa, making them a model system for understanding the geographical distribution of amphibians across the continent.

Amphibians are widely distributed in Africa [22,34,71,72]. Their high species richness in Central Africa, and particularly in the DRC, confirms the hypothesis that small vertebrates form a majority across a wide range of habitats [73]. This specific richness varies with the level of endemism specific to each country [1,23], altitude [74–76], latitude [77] and climate [78,79] that characterize each study area. In the DRC, for example, this variation is the result of the ecological conditions of the habitats [80], with which the Congo River and its tributaries are associated, including effective ecological barriers that have a considerable impact on species distribution [16,81,82]. Studies carried out to date in the DRC fall far short of the number of species known there [23] and, in the Afro-tropical region, place the country second only to Madagascar [2]. The protected status of many Congolese forests and their inaccessibility could explain why this type of study is rather rare.

Despite their distribution in Africa, amphibians are still largely ignored [83], particularly in the DRC [7,16]. This ignorance, difficult habitat conditions and differences in methodological surveys are the probable explanations for the differences in specific richness observed between different studies. The similarities observed between the studies, followed by their groupings, are linked to their similar specific richness and composition. This is reflected in the geographical location of the study areas (Figure 7) that allows species to circulate with each other. Examples include the YFRE, the Okapi Wildlife Reserve and the Kokolopori Reserve in the DRC. The same applies to the areas surveyed in Gabon and Cameroon. On the other hand, the Lokutu Forest and the Bas-Congo region in the DRC are similar to the Nouabale-Ndoki National Park and the Lac Télé Community Reserve in the Republic of the Congo. According to [19], this may be explained by the ability of some species to cross the geographical barriers between the two countries.

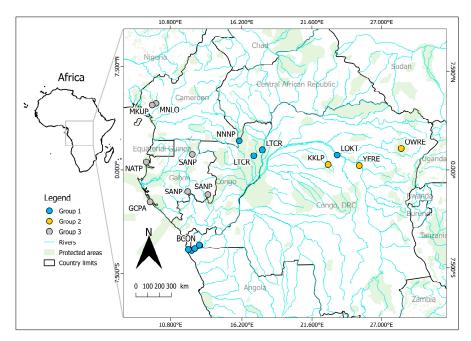


Figure 7. Location map of study areas used for comparison in Central Africa. Group 1: Bas-Congo, Lokutu, Lake Télé Community Reserve, Nouabale-Ndoki National Park; Group 2: Kokolopori, Okapi Wildlife Reserve, Yoko Forest Reserve; Group 3: Gamba Complex of Protected Areas, Mount Kupe, Mount Nlonako, National Parks, Sites Adjacent to three National Parks. Data sources: protected areas and rivers layers (COMIFAC: https://www.observatoire-comifac.net/publications/library\_gis? lang=en&page=9, accessed on 21 March 2024), countries layer (RGC: https://www.rgc.cd, accessed on 21 March 2024).

Amphibians in the DRC are divided into two orders, Gymnophiona and Anura [1,22]. The absence of Gymnophiona in the YFRE, as in Kokolopori [16], may be linked to their capture techniques, which are different from those of Anurans, based on their burrowing habits [53,84]. The amphibian fauna in the YFRE consist only of Anurans, which are widely distributed in Congolese forests [14,16]. Among the species known to be endemic to the DRC [22,23], Afrixalus equatorialis, Hyperolius langi, Hyperolius parallelus and Ptychadena christyi are present in the YFRE. A. equatorialis also lives in the Lokutu forest [13]; A. equatorialis, H. langi and P. christyi are distributed in the Kokolopori forest [16]; and H. langi and P. christyi are known from the Okapi Wildlife Reserve [48]. H. parallelus is present in Bas-Congo [14]; A. equatorialis and P. christyi have been reported in Kisangani [85]. A recent study by [11] indicated the absence of *Hyperolius langi* in Kokolopori. The distribution of these four species in these areas suggests their endemism in the DRC. Alongside these species, other endemic species have been identified by various studies. These are Arthroleptis phrynoides [16], Hemisus olivaceus, Hyperolius ferrugineus, Ptychadena ingeri [48] and Hymenochirus boulengeri [16,48]. The number of endemic species recognized by studies carried out in the DRC, as in other Central African countries, is thought to be linked to the persistence of fluvial forest refugia in various environments [86,87].

The presence of amphibians in the YFRE is confirmed by the geographical distribution of the various species provided in the literature [1,11,19,23,31,33,36,37]. The number of amphibian predators and prey in the YFRE is thought to be declining as a result of unregulated hunting by local people in the surrounding villages. The location of this Reserve along the Kisangani–Ubundu road and its contact with the Congo River are likely factors that expose it to licit or illicit exploitation of its resources. These factors impact habitat conditions [88,89], which in turn affect amphibian populations [90–93]. Despite the current anthropogenic pressure on ecosystems, the species recorded in the YFRE demonstrate that there are still fragments of undisturbed primary forest around the city of Kisangani.

## 5. Conclusions

This study provided baseline data to deepen our knowledge of amphibian ecology in the YFRE, located in the DRC's most diverse region. The results showed that the amphibian fauna in this part of the country is represented by 33 species, namely Amietia nutti, Amnirana albolabris, Phrynobatrachus auritus, P. perpalmatus, Leptopelis calcaratus, Leptopelis ocellatus, L. notatus, L. millsoni, L. christyi, Arthroleptis tuberosus, A. variabilis, Sclerophrys gracilipes, S. gutturalis, S. pusilla, Nectophryne batesii, Hyperolius sp., H. langi, H. ocellatus, H. parallelus, H. platyceps, Afrixalus quadrivittatus, A. equatorialis, A. osorioi, Ptychadena perreti, P. mascareniensis, P. christyi, Hoplobatrachus occipitalis, Cryptothylax greshoffi, Chiromantis rufescens, Cardioglossa leucomystax, Kassina maculosa, Hylambates verrucosus and Xenopus pygmaeus. Hyperoliidae and Arthroleptidae families are richer in species than all other families, with Ranidae being the most abundant. Amnirana albolabris is the most abundant species. From the presence of these species, many other aspects can be studied. These include assessing the effects of natural habitat modifications on amphibian populations. This involves the response of species to the diversity of habitats resulting from fragmentation. Understanding this anthropogenic pressure on natural habitats will require preservation and sustainable management, which are beneficial to the diversity of zoological groups such as amphibians.

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**Institutional Review Board Statement:** This study was not subject to ethical review and approval as the field data collection methodology used the live capture of animals. Captured animals were marked and released. This allows the population to be re-sampled later without any bias using the same method, which avoids the destruction of the animals and facilitates the maintenance of ecological processes within the habitats.

**Data Availability Statement:** The data provided are confidential and available on request from Loving Musubaho and Jan Bogaert.

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#### References

- 1. Frost, D.R. *Amphibian Species of the World: An Online Reference*; Version 6.2; American Museum of Natural History: New York, NY, USA, 2024. Available online: https://amphibiansoftheworld.amnh.org/index.php (accessed on 24 May 2024).
- 2. Chanson, J.; Hoffmann, M.; Cox, N.; Stuart, S. The state of the world's amphibians. In *Threatened Amphibians of the World*, 1st ed.; Stuart, S.N., Hoffmann, M., Chanson, J.S., Cox, N.A., Berridge, R.J., Ramani, P., Young, B.E., Eds.; Lynx Edicions: Barcelona, Spain, 2008; pp. 33–52.
- 3. Dargie, G.C.; Lawson, I.T.; Rayden, T.J.; Miles, L.; Mitchard, E.T.A.; Page, S.E.; Bocko, Y.E.; Ifo, S.A.; Lewis, S.L. Congo Basin peatlands: Threats and conservation priorities. *Mitig. Adapt. Strateg. Glob. Chang.* **2019**, 24, 669–686. [CrossRef]
- 4. Shapiro, A.C.; Grantham, H.S.; Anguilar-Amuchastegui, N.; Murray, N.J.; Gond, V.; Bonfils, D.; Rickenbach, O. Forest condition in the Congo Basin for the assessment of ecosystem conservation status. *Ecol. Indic.* **2021**, *122*, 107268. [CrossRef]
- 5. Greenbaum, E. *Emerald Labyrinth: A Scientist's Adventures in the Jungles of the Congo*; ForeEdge (Imprint of University Press of New England): Lebanon, NH, USA, 2017; p. 329.
- 6. Ribeiro, J.W.; Siqueira, T.; Brejāo, G.L.; Zipkin, E.F. Effects of agricultural and topography on tropical amphibian species and communities. *Ecol. Appl.* **2018**, *28*, 1554–1564. [CrossRef] [PubMed]
- 7. Chifundera, K.Z. Using diversity indices for identifying the priority sites for herpetofauna conservation in the Democratic Republic of the Congo. *Nat. Conserv. Ress.* **2019**, *4*, 13–33. [CrossRef]
- 8. Van der Hoek, Y.; Tuyisingize, D.; Eckardt, W.; Garriga, N. Spatial variation in anuran richness, diversity and abundance across montane wetland habitat in Volcanoes National Park, Rwanda. *Ecol. Evol.* **2019**, *9*, 4220–4230. [CrossRef] [PubMed]
- 9. Anjos, A.G.; Costa, R.N.; Brito, D.; Solé, M. Is there an association between the ecological characteristics of anurans from the Brazilian Atlantic Forest and their extinction risk? *Ethol. Ecol. Evol.* **2020**, *32*, 336–350. [CrossRef]
- 10. Astruc, G.; Miaud, C.; Besnard, A.; Barrioz, M. Le déclin alarmant des amphibiens de France: l'exemple étayé de la Normandie. *Bull. Soc. Herp. Fr.* **2021**, *178*, 57–74. [CrossRef]
- 11. Schiøtz, A. Notes on the genus Hyperolius (Anura, Heproliidae) in Central Democratic Republic of Congo. Alytes 2006, 24, 40–60.
- 12. Larson, T.R.; Castro, D.; Behangana, M.; Greenbaum, E. Evolutionary history of the river frog genus Amietia (Anura: Pyxycephalidae) reveals extensive diversification in Central African highlands. *Mol. Phyl. Evol.* **2016**, *99*, 168–181. [CrossRef]
- 13. Penner, J.; Rödel, M.-O. Amphibians and reptiles of Lokutu. In *A Rapid Biological Assessment of Lokutu, Democratic Republic of Congo*; Butynski, T.M., McCullough, J., Eds.; RAP Bulletin of Biological Assessment 46: Arlington, VA, USA, 2007; pp. 37–41.
- 14. Nagy, Z.T.; Kusamba, C.; Collet, M.; Gvoždík, V. Notes on the herpetofauna of western Bas-Congo, Democratic Republic of Congo. *Herpetol. Notes* **2013**, *6*, 413–419.
- 15. Hirschfeld, M.; Blackburn, D.C.; Burger, M.; Greenbaum, E.; Zassi-Boulou, A.G.; Rödel, M.-O. Two new species of Long-fingered Frogs of the genus Cardioglossa (Anura: Arthroleptidae) from central African rainforests. *Afr. J. Herpetol.* **2015**, *64*, 81–102. [CrossRef]
- 16. Badjedjea, G.; Masudi, F.M.; Akaibe, B.D.; Gvoždík, V. Amphibians of Kokolopori: An introduction to the amphibian fauna of the Central Congolian Lowland Forests, Democratic Republic of the Congo. *Amph. Rept. Conserv.* **2022**, *16*, 35–70.
- 17. DeJong, T.M. A comparison of three diversity indices based on their components of richness and evenness. *Oikos* 1975, 26, 222–227. [CrossRef]
- 18. Magurran, A.E. Measuring Biological Diversity; Blackwell Publishing Company: Oxford, UK, 2004; pp. 1–256.
- 19. Jongsma, G.F.M.; Barej, M.F.; Barrat, C.D.; Burger, M.; Conradie, W.; Ernst, R.; Greenbaum, E.; Hirschfeld, M.; Leaché, A.D.; Penner, J.; et al. Diversity and biogeography of frogs in the genus Amnirana (Anura: Ranidae) across sub-Saharan Africa. *Mol. Phyl. Evol.* 2018, 120, 274–285. [CrossRef] [PubMed]
- 20. Quérouil, S. Intérêts et Limites de L'approche Moléculaire Pour Aborder la Biogéographie et la Spéciation: Quelques Exemples Chez Les Mammifères d'Afrique Tropicale. Ph.D. Thesis, Université Rennes, Paimpont, France, 2001.

21. Nicolas, V. Systématique et Ecologie Des Communautés Tropicales Des Muridés (Mammalia, Rodentia) et Des Soricidés (Mammalia, Insectivora). Ph.D. Thesis, Université Rennes, Paimpont, France, 2003.

- 22. Frétey, T.; Blanc, C.P. Liste Des Amphibiens d'Afrique Centrale: Cameroun, Congo, Gabon, Guinée Equatoriale, République Centrafricaine, République Démocratique du Congo, São Tomé et Principe; Association pour le Développement de l'Information Environnementale (ADIE): Libreville, Gabon, 2000; pp. 1–38.
- 23. Frétey, T.; Dewynter, M.; Blanc, C.P. *Amphibiens d'Afrique Centrale et d'Angola. Clé de Détermination Illustrée des Amphibiens du Gabon et du Mbini*; Illustrated identification key of the amphibians from Gabon and Mbini; Editions Biotope; Muséum national d'Histoire naturelle: Paris, France, 2011; pp. 1–232.
- 24. Boyemba, B.F. Ecologie de Pericopsis Elata (Harms) Van Meeuwen (Fabaceae), Arbre de Forêt Tropicale Africaine à Répartition Agrégée. Ph.D. Thesis, Université Libre de Bruxelles, Bruxelles, Belgique, 2011.
- 25. Picard, N.; Boyemba, F.; Rossi, V. Reducing the error in biomass estimates strongly depends on model selection. *Ann. for. Sci.* **2015**, 72, 811–823. [CrossRef]
- 26. Kahindo, M.J.-M. Potentiel en Produits Forestiers Autres Que le Bois D'œuvre Dans Les Formations Forestières de la Région de Kisangani. Cas Des Rotins Eremospatha haullevilleana de Wild. et Laccosperma secundiflorum (P. Beauv.) Kuntze de la Réserve Forestière de Yoko (Province Orientale, RD Congo). Ph.D. Thesis, Université de Kisangani, Kisangani, Democratic Republic of the Congo, 2011.
- Sabongo, Y. Etude Comparative de la Structure et de la Diversité Des Forêts à Gilbertiodendron dewevrei (De Wild.) J. Léonard Des Régions de Kisangani et de l'Ituri (RDC). Ph.D. Thesis, Université de Kisangani, Kisangani, Democratic Republic of the Congo, 2015.
- 28. Veith, M.; Lötters, S.; Andreone, F.; Rödel, M.-O. Measuring and monitoring amphibian diversity in tropical forests. II. Estimating species richness from standardized transect censing. *Ecotropica* **2004**, *10*, 85–99.
- 29. Heyer, W.R.; Donnelly, M.A.; McDiarmid, R.W.; Hayek, L.-A.C.; Foster, M.S. *Measuring and Monitoring Biological Diversity, Standard Methods for Amphibians*; Smithsonian Institution Press: London, UK; Paris, France, 1994; pp. 1–364.
- 30. Maynou, X.; Martin, R.; Aranda, D. The role of small secondary biotopes in a highly fragmented landscape as habitat and connectivity providers for dragonflies (insecta: Odonata). *J. Insect Conserv.* **2017**, 21, 517–530. [CrossRef]
- 31. Schiøtz, A. The Treefrogs of Africa; Chimaira: Frankfurt am Main, Germany, 1999; pp. 1-350.
- 32. Channing, A. *Amphibians of Central and Southern Africa*; Comstock Publishing Associations: Ithaca, NY, USA; London, UK; Paris, France, 2001; pp. 1–455.
- 33. Channing, A.; Howell, K.M. Amphibians of East Africa; Chimaira: Frankfurt am Main, Germany, 2006; pp. 1–403.
- 34. Howell, K.M. Field Guide to the Amphibians of the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya; CPI: Nairobi, Kenya, 2010; pp. 1–316.
- 35. Channing, A.; Rödel, M.-O. Field Guide to the Frogs and Other Amphibians of Africa; Struik Nature: Cape Town, South Africa, 2019; pp. 1–408.
- 36. Dewynter, M.; Frétey, T. Liste taxonomique commentée et catalogue illustrée des amphibiens du Gabon. *Les Cah. De La Fond. Biot.* **2019**, 27, 2–84.
- 37. Dehling, J.M.; Sinsch, U. Amphibians of Rwanda: Diversity, community features and conservation status. *Diversity* **2023**, 15, 512. [CrossRef]
- 38. Edgar, R.C. Accuracy of microbial community diversity estimates by closed- and open-reference OTUs. *PeerJ* **2017**, *5*, e3889. [CrossRef]
- 39. Weiss, S.; Xu, Z.Z.; Peddada, S.; Amir, A.; Bittinger, K.; Gonzalez, A.; Lozupone, C.; Zaneveld, J.R.; Vázquez-Baeza, Y.; Birmingham, A.; et al. Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* 2017, 5, 27. [CrossRef] [PubMed]
- 40. Boussarie, G.; Bakker, J.; Wangensteen, O.S.; Mariam, S.; Bonnin, L.; Juhel, J.-B.; Kiszka, J.J.; Kulbicki, M.; Manel, S.; Robbins, W.D.; et al. Environmental DNA illuminates the dark diversity of sharks. *Sci. Adv.* **2018**, *4*, eaa99661. [CrossRef] [PubMed]
- 41. Mindje, M.; Tumushimire, L.; Sinsch, U. Diversity assessment of anurans in the Mugesera wetland (eastern Rwanda): Impact of habitat disturbance and partial recovery. *Salamandra* **2020**, *56*, 27–38.
- 42. Légendre, P.; Légendre, L. Numerical Ecology, 3rd ed.; Elsevier: Amsterdam, The Netherlands, 2012; pp. 1–990.
- 43. Borcard, D.; Gillet, F.; Legendre, P. *Numerical Ecology with R*, 2nd ed.; Springer International Publishing AG: Berlin/Heidelberg, Germany, 2018; pp. 1–435.
- 44. R Core Team. *R: A Language and Environment for Statistical Computing*; R Version 4.3.1; R Foundation for Statistical Computing: Vienna, Austria, 2023. Available online: https://www.R-project.org/ (accessed on 28 September 2023).
- 45. Oksanen, J.; Simpson, G.L.; Blanchet, F.G.; Kindt, R.; Legendre, P.; Minchin, P.R.; O'Hara, R.B.; Solymos, P.; Stevens, M.H.H.; Szoecs, E.; et al. Vegan: Community Ecology Package. R Package Version 2.6-4. 2022. Available online: https://CRAN.R-project.org/package=vegan (accessed on 15 November 2023).
- 46. Kassambara, A.; Mundt, F. Factoextra: Ectract and Visualize the Results of Multivariate Data Analyses. R Package Version 1.0.7. 2020. Available online: https://rpkgs.datanovia.com/factoextra/index.html (accessed on 19 January 2024).
- 47. Kindt, R.; Coe, R. *Tree Diversity Analysis. A Manual and Software for Common Statistical Methods for Ecological and Biodiversity Studies*; World Agroforestry Centre (ICRAF): Nairobi, Kenya, 2005; pp. 1–196.

48. Masudi, F.M.; Katuala, P.G.B.; Chifundera, Z.K.; Badjedjea, G.B.; Kambili, J.-R.S.; Lotana, A.L.; Ewango, C.; Gembu, G.-C.T.; Akaibe, D. Preliminary data on amphibian diversity of the Okapi Wildlife Reserve (RFO) in Democratic Republic of the Congo. *Am. J. Zool.* **2019**, *2*, 38–43.

- 49. Herrmann, H.W.; Böhme, W.; Hermann, P.A.; Plath, M.; Schmitz, A.; Solbach, M. African biodiversity hotspots: The amphibians of Mt Nlonako, Cameroon. *Salamandra* **2005**, *41*, 61–81.
- 50. Portik, D.M.; Jongsma, G.F.M.; Kouete, M.T.; Scheinberg, L.A.; Freiermuth, B.; Tapondjou, W.P.; Blackburn, D.C. A survey of amphibians and reptiles in the foothills of Mount Kupe, Cameroon. *Amph. Rept. Conserv.* **2016**, *10*, 37–67.e131.
- 51. Jackson, K.; Blackburn, D.C. The amphibians and reptiles of Nouabale-Ndoki National Park, Republic of Congo (Brazzaville). *Salamandra* **2007**, *43*, 149–164.
- 52. Jackson, K.; Zassi-Boulou, A.-G.; Mavoungou, L.-B.; Pangou, S. Reptiles and Amphibians of the Lac Télé Community Reserve, Likouala Region, Republic of Congo (Brazzaville). *Herp. Conserv. Biol.* **2007**, 2, 75–86.
- 53. Burger, M.; Pauwels, O.S.G.; Branch, W.R.; Tobi, E.; Yoga, J.A.; Mikolo, E.N. An assessment of the amphibian fauna of the Gamba Complex of Protected Areas, Gabon. In *Biodiversity of an Equatorial African Rainforest*; Alonso, A., Lee, M.E., Campebell, P., Pauwels, O.S.G., Et Dallmeier, F., Eds.; Bulletin of the Biological Society of Washington: Washington, DC, USA, 2006; pp. 297–307.
- 54. Pauwels, O.S.G.; Rödel, M.-O. Amphibians and National Parks in Gabon, Western Central Africa. Herpetozoa 2007, 19, 135-148.
- 55. Larson, J.G.; Zimkus, B.M. Preliminary assessment of the frog assemblages from sites adjacent to three National Parks in Gabon. Herp. Conserv. Biol. 2018, 13, 240–256.
- 56. Aliko, N.G.; Assemian, N.E.; Boussou, K.C.; Keita, G.; Konan, K.F. Habitat-based breeding strategies of female Hoplobatrachus occipitalis (Anura: Dicroglossidae) from Daloa department, Midwest of Côte-d'Ivoire. *Intern. J. Res. Stud. Zool.* **2018**, *4*, 28–36.
- 57. Tlidjane, A.; Menaa, M.; Rebbah, A.C.; Tellailia, S.; Seddik, S.; Chefrour, A.; Maazi, M.C. La richesse et la distribution des amphibiens dans la région de Souk Ahras (Nord-Est de l'Algérie). *Bull. Soc. Zool. Fr.* **2019**, 144, 179–201.
- 58. Bittencourt-Silva, G.B. Herpetological survey of western Zambia. Amph. Rept. Conserv. 2019, 13, 1–28.e181.
- 59. Boissinot, A.; Besnard, A.; Lourdais, O. Amphibian diversity in farmlands: Combined influences of breeding-site and landscape attributes in Western France. *Agric. Ecosyst. Env.* **2019**, *269*, 51–61. [CrossRef]
- 60. Amiet, J.-L. Ecologie et distribution des amphibiens Anoures de la région de Nkongsamba (Cameroun). *Ann. Fac. Sc. Yaoundé* 1975, 20, 33–107.
- 61. Amiet, J.-L. Les amphibiens Anoures de la région de Mamfé (Cameroun). Ann. Fac. Sc. Yaoundé 1978, 25, 189-219.
- 62. Pages, J.M. Ecologie des amphibiens du Sud-Ouest de la montagne Noire, France. Alytes 1984, 3, 56-59.
- 63. Mukinzi, I.; Katuala, G.B.; Kennis, J.; Gambalemoke, M.; Kadange, N.; Dudu, A.M.; Colyn, M.; Hutter, R. Preliminary data on the biodiversity of rodents and insectivores (Mammalia) in the periphery of Kisangani (DR Congo). *Belg. J. Zool.* **2005**, *135* (Suppl. 1), 133–140.
- 64. Fournier, A.; Floret, C.; Gnahoua, G.-M. Végétation des jachères et succession post-culturale en Afrique tropicale. In *La Jachère en Afrique Tropicale*; Floret, C., Pontanier, R., Eds.; John Libbey Eurotext: Paris, France, 2001; pp. 123–168.
- 65. Isingoma, J.; Sande, E.; Kityo, R.; Hughes, D.F. Amphibian communities along a forest degradation gradient in an East African forest reserve. *Ecol. Inform.* **2023**, *75*, 102021. [CrossRef]
- 66. Wells, K.D. The Ecology and Behaviour of Amphibians; Chicago University Press: Chicago, IL, USA, 2007; pp. 1–1162.
- 67. Iyongo, W.M.; Visser, M.; Leirs, H.; Iyongo, B.; Ulyel, A.; Bogaert, J. Etude préliminaire des effets de la fragmentation des forêts sur la similarité des habitats et leurs richesses en espèces de rongeurs (Masako, RD Congo). *Ann. ISEA* **2009**, *4*, 177–186.
- 68. Onadeko, A.B. Distribution, diversity and abundance of anuran species in three vegetation habitats in Southwestern Nigeria. *Eth. J. Env. Stud. Manag.* **2016**, *9*, 22–34. [CrossRef]
- 69. Perret, J.L. Les amphibiens du Cameroun. Zool. Jahrb. Jenn. Abt. F Syst. 1966, 8, 289–464.
- 70. Inger, R.F. *Amphibia in Exploration Parc National de la Garamba*; Fascicule 52: Kinshasa, Democratic Republic of the Congo, 1968; p. 190.
- 71. Rödel, M.-O. Herpetofauna of West Africa 1. Amphibians of the West African Savana; Chimaira: Frankfurt am Main, Germany, 2000; pp. 1–332.
- 72. Blackburn, D.C. Biogeography and evolution of body size and life history of African frogs: Phylogeny of Squeakers (Arthroleptis) and long-fingered frogs (Cardioglossa) estimated from mitochondrial data. *Mol. Phyl. Evol.* **2008**, *49*, 806–826. [CrossRef] [PubMed]
- 73. Amrouche-Larabi, L.; Denys, C.; Boukhemza, M.; Bensidhoum, M.; Hamani, A.; Nicolas, V.; Khifer, L.; Mamou, R. Inventaire des petits vertébrés terrestres de quelques localités du nord Algérien. *Trav. De L'inst. Scient.* **2015**, *8*, 85–95.
- 74. Koirala, B.K.; Cheda, K.; Penjor, T. Species diversity and spatial distribution of amphibian fauna along the altitudinal gradients in Jigme Dorji Natonal Park, Western Bhutan. *J. Thr. Taxa* **2019**, *11*, 14249–14258. [CrossRef]
- 75. Villacampa, J.; Whitworth, A.; Alle, L.; Malo, J. Altitudinal differences in alpha, beta and functional diversity of an amphibian community in a biodiversity hotspot. *Neotrop. Biodiv.* **2019**, *5*, 60–68. [CrossRef]
- 76. Willig, M.R.; Presley, S. Biodiversity and metacommunity structure of animals along altitudinal gradients in tropical montane forests. *J. Trop. Ecol.* **2016**, 32, 421–436. [CrossRef]
- 77. Zhang, L.; Lu, X. Amphibians live longer at higher altitudes but not at higher latitudes. *Biol. J. Lin. Soc.* **2012**, *106*, 623–632. [CrossRef]

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78. Ryan, M.J.; Fuller, M.M.; Scott, N.J.; Cook, J.A.; Poe, S.; Willink, B.; Chaves, G.; Bolaňos, F. Individualistic population responses of five frog species in two changing tropical environments over time. *PLoS ONE* **2014**, *9*, e98351.

- 79. Ryan, M.J.; Scott, N.J.; Cook, J.A.; Willink, B.; Chaves, G.; Bolaňos, F.; García-Rodríguez, A.; Latella, I.M.; Koerner, S.E. Too wet frogs: Changes in a tropical leaf litter community coincide with La Niňa. *Ecosphere* **2015**, *6*, 4. [CrossRef]
- 80. Vagmaker, N.; Pereira-Ribeiro, J.; Ferreguetti, Á.C.; Boazi, A.; Gama-Matos, R.; Bergallo, H.G.; Rocha, C.F.D. Structure of the leaf litter frog community in an area of Atlantic Forest in Southeastern Brazil. *Zoologia* **2020**, *37*, e38877. [CrossRef]
- 81. Gascon, C.; Malcolm, J.R.; Patton, J.L.; da Silva, M.N.F.; Bogart, J.P.; Lougheed, S.C.; Peres, C.A.; Neckel, S.; Boag, P.T. Riverine barriers and the geographic distribution of Amazonian species. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13672–13677. [CrossRef]
- 82. Flügel, T.J.; Eckardt, F.D.; Cotterill, F.P.D. The present-day drainage patterns of the Congo River system and their Neogene evolution. In *Geology and Potential of the Congo Basin*; De Wit, M.J., Guillocheau, F., De Wit, M.C.J., Eds.; Springer: Heidelberg/Berlin, Germany, 2015; pp. 315–337.
- 83. Lawson, D.P.; Klemens, M.W. Herpetofauna of the African Rain Forest: Overview and Recommendations for Conservation. In *African Rainforest Ecology and Conservation*; Weber, W., White, L.J.T., Vedder, A., et Naughton-Treves, L., Eds.; Yale University Press: New Haven, CT, USA, 2001; pp. 291–307.
- 84. Howell, K.M. Les herptiles: Amphibiens et reptiles. In *La Biodiversité Des Forêts d'Afrique: Manuel Pratique de Recensement Des Vertébrés*, 2nd ed.; Davies, G., Hoffmann, M., Eds.; Earthwatch Institute Europe: Newton, MA, USA, 2004; pp. 19–48.
- 85. Musubaho, K.W.L.; Iyongo, W.M.; Ilonga, M.B.; Mapoli, M.J.; Mbumba, J.L.J.; Neema, S.M.; Tungaluna, G.C.G.; Mukinzi, J.C.I.; Bogaert, J. Méta-analyse exploratoire des effets de perturbations anthropiques sur la diversité des Amphibiens dans les stations de Kasugho, Butembo, Mambasa et Kisangani en République Démocratique du Congo. *Tropicultura* **2021**, *39*, 1709.
- 86. Plana, V. Mechanisms and tempo of evolution in the African Guineo-Congolian rainforest. *Phil. Trans. Roy. Soc. Lond. B* **2004**, 359, 1585–1594. [CrossRef]
- 87. Leaché, A.D.; Portik, D.M.; Rivera, D.; Rödel, M.-O.; Penner, J.; Gvoždík, V.; Greenbaum, E.; Jongsma, G.F.M.; Ofori-Boateng, C.; Buger, M.; et al. Exploring rainforest diversification using demographical model testing in the African Foam-nest Treefrog, Chiromantis rufescens. J. Biog. 2019, 46, 2706–2721. [CrossRef]
- 88. Haddad, N.M.; Brudvig, L.A.; Clobert, J.; Davies, K.F.; González, A.; Holt, R.D.; Lovejoy, T.E.; Sexton, J.O.; Austin, M.P.; Collins, C.D.; et al. Habitat fragmentation and its lasting impact on Earth's ecosystems. *Sci. Adv.* 2015, 1, e1500052. [CrossRef] [PubMed]
- 89. Newbold, T.; Hudson, L.N.; Hill, S.L.L.; Contu, S.; Lysenko, I.; Senior, R.A.; Börger, L.; Bennett, D.J.; Choimes, A.; Collen, B.; et al. Global effects of land use on local terrestrial biodiversity. *Nature* **2015**, *520*, 45–50. [CrossRef] [PubMed]
- 90. Figueiredo, G.T.; Storti, L.F.; Lourenco-De-Moraes, R.; Shibatta, O.A.; Anjos, L. Influence of microhabitat on the richness of anuran species: A case study of different landscapes in the Atlantic Forest of southern Brazil. *An. Da Acad. Bras. De Ciências* **2019**, 91, e20171023. [CrossRef]
- 91. Decena, S.C.P.; Avorque, C.A.; Decena, I.C.P.; Asis, P.D.; Pacle, B. Impact of habitat alteration on amphibian diversity and species composition in a lowland tropical rainforest in Northeastern Leyte, Philippines. *Sci. Rep.* **2020**, *10*, 10547. [CrossRef]
- 92. Sykes, L.; Santini, L.; Etard, A.; Newbold, T. Effects of rarity form on species' responses to land use. *Conserv. Biol.* **2020**, *34*, 688–696. [CrossRef]
- 93. Bellotto-Trigo, F.C.; Uezu, A.; Hatfield, J.H.; Morante-Filho, J.C.; dos Anjos, L.; Develey, P.F.; Clegg, T.; Orme, C.D.L.; Banks-Leite, C. Intraspecific variation in sensitivity to habitat fragmentation is influenced by forest cover and distance to the range edge. *Biol. Conserv.* 2023, 284, 110167. [CrossRef]

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