



# Preliminary Data on Amphibian Diversity of the Okapi Wildlife Reserve (RFO) in Democratic Republic of the Congo

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**Abstract:** Amphibians are poorly known in the Okapi Wildlife Reserve (RFO) in DRC, and amphibians are identified as one of the most threatened animal taxa among vertebrates living on Earth. The aim of this study was to inventory amphibians in the Okapi Wildlife Reserve. To do this, amphibians were caught by hand during the day (between 06:00–08:00 hrs) and at night (between 18:00–20:00 hrs). All specimens were photographed, labelled, and preserved in ethanol (70%). Specimens were located by sight and sound. In two sessions of 10 days each, 692 specimens were caught, representing 53 species, 17 genera and 11 families. All the inventoried species belong to the Order Anura. Several specimens could not be identified to species. The family representation included Hyperoliidae (159 specimens: 22.97%), Pyxicephalidae (8 specimens (1.15%)), Arthroleptidae (54 individuals: 7.80%), Rhacophoridae (2 specimens: 0.28%), Hemisotidae (34 specimens: 4.91%), Dicroglossidae (123 specimens: 17.77%), Ranidae (174 individuals: 25.14%), Phrynobatrachidae (3 individuals: 0.43%), Ptychadenidae (22 specimens: 3.17%), Bufonidae (45 specimens: 6.5%) and Pipidae (68 individuals: 9.82%). The results of this research are preliminary, but they are very interesting because they will allow the Reserve authorities to know the amphibians of the RFO and to have a scientific basis for a possible drafting or implementation of the conservation plan and the protection of wetlands.

**Keywords:** Biodiversity, Amphibians, Habitat Loss, Okapi Wildlife Reserve, Ituri Forest, Epulu, DRC

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## 1. Introduction

Natural history and field ecology are essential for the effective conservation and management of herpetofauna [1]. Amphibians have long been part of human culture [2]. Currently, these amphibians are identified as one of the most threatened animal taxa among vertebrates living on Earth, because about 41% of them are at risk of extinction due mainly to human activities and fungal diseases worldwide [3-5]. This disease that kills amphibians is due to a fungus species: *Batrachochytrium dendrobatidis* [6] whose recent origin is Asia [7]. This fungus is now known in several African countries, namely Gabon, Benin, DRC north of ex-Katanga [5, 6, 10]. Apart from the disease, bushfire, known mainly in savannah regions and the effects of climate change, are added to the list of causes of amphibian loss [12].

The Okapi Wildlife Reserve (RFO) is located in a confluence zone, a forest refuge rich in exceptional (rare and threatened) and emblematic biodiversity. But amphibians are poorly studied in this Reserve. The purpose of this study was to inventory the amphibians of the RFO, highlight its diversity, and contribute to the knowledge of amphibians in this reserve of national and international interest, which is currently at risk.

## 2. Materials and Methods

### 2.1. Area of Study

The RFO consists of a unique ecosystem as a Pleistocene refuge dominated by a dense evergreen forest of *Gilbertiodendron dewevrei* and a humid forest that intertwines with swampy forests located along watercourses, with granitic inselbergs that shelter several endemic species such as the Giant Cycad (*Encephalartos ituriensis*) [14]. Two sessions were conducted to collect biological materials, in the RFO and in Mambasa. We sampled in July 2009 and from 22 to 05 August 2015. Each capture session was 10 days long. All geographic coordinates were collected by a GARMIN map 60CSx GPS.

The RFO is located in the northeast of the Democratic Republic of the Congo (DRC), in Ituri Province, in the Ituri district. It lies between 1° and 2°29' from the north latitude and 28° and 29°4' from the east longitude. Its altitude is

between 700 m and 1000 m. It covers an area of 13,726 km<sup>2</sup>. A total of 90% of its area is located in the territory of Mambasa, 7% in the territory of Wamba and 3% in the territory of Watsa. The RFO was created by Ministerial Order N°045/CM/ECN/92 of 2 May 1992, and was included in UNESCO's World Heritage List in 1996 under criterion X [13]. For the time being, it is classified as a UNESCO world heritage site in danger in 1997.



Figure 1. Presentation of some amphibian capture sites in RFO.

### 2.2. Data Collection Methods

We selected the capture site during the day. Each habitat was photographed using a Nikon Coolpix AW 130 camera. After the photography, we returned to the site. At night, we would leave for the amphibian catches. The capture was done by hand. To do this, specimens were located visually and by ear between 6pm. and 10pm., the time when most species are active in search of either food or a sexual partner [15]. This capture technique was effective for all species because habitats were chosen on the basis of their ability to shelter amphibians. All specimens were labelled, mounted in a plastic stocking containing the formalin (10%), and remained in place for 24 hours. They were then transferred to ethanol (70%) for permanent preservation. Necropsies consisted of muscle tissue, stored in Eppendorf tubes containing alcohol (75%). Molecular analyses of unidentified species up to the species are underway at the University of Texas at El Paso (USA).

## 3. Results

### 3.1. Amphibian Diversity in the RFO

Table 1. List of amphibians caught in the Ituri Forest (RFO).

Family	Genus	Species	UICN Status	Total	%
Hyperoliidae Laurent, 1943	<i>Afrivalus</i> Laurent, 1944	<i>Afrivalus osorioi</i> (Ferreira, 1906)	LC	1	0,14
	<i>Cryptothylax</i> Laurent et Combaz, 1950	<i>Cryptothylax greshoffii</i> (Schilthuis, 1899)	LC	1	0,14
	<i>Hyperolius</i> Rapp, 1842	<i>Hyperolius bolifambae</i> Mertens, 1938	LC	5	0,72
		<i>Hyperolius cf brachiofasciatus</i> Ahl, 1931	DD	1	0,14
		<i>Hyperolius cinnamomeoventris</i> Bocage, 1866	LC	56	8,09
		<i>Hyperolius ferrugineus</i> Laurent, 1943	DD	3	0,43
		<i>Hyperolius kuligae</i> Mertens, 1940	LC	8	1,16
		<i>Hyperolius langi</i> Noble, 1924	LC	53	7,66

Family	Genus	Species	UICN Status	Total	%
		<i>Hyperolius lateralis</i> Laurent, 1940	LC	2	0,29
		<i>Hyperolius nasutus</i> Günther, 1865	LC	15	2,17
		<i>Hyperolius ocellatus</i> Gunther, 1858	LC	6	0,87
		<i>Hyperolius platyceps</i> (Boulenger, 1900)	LC	1	0,14
		<i>Hyperolius sp</i>		5	0,72
	Kassina Girard, 1853	<i>Kassina senegalensis</i> (Duméril & Bibron, 1841)	LC	1	0,14
	Phlyctimantis Laurent et Combaz, 1950	<i>Phlyctimantis verrucosus</i> (Boulenger, 1912)	LC	1	0,14
Pyxicephalidae Bonaparte, 1850	Amietia Dubois, 1987	<i>Amietia chapini</i> (Noble, 1924)	LC	1	0,14
		<i>Amietia angolensis</i> (Bocage, 1866)	LC	7	1,01
	Arthroleptis Smith, 1849	<i>Arthroleptis sp</i>		3	0,43
	Leptopelis Günther, 1859	<i>Leptopelis christyi</i> (Boulenger, 1912)	LC	3	0,43
Arthroleptidae Mivart, 1869		<i>Leptopelis ocellatus</i> (Mocquard, 1902)	LC	7	1,01
		<i>Leptopelis notatus</i> (Peters, 1875)	LC	2	0,29
		<i>Leptopelis calcaratus</i> (Boulenger, 1906)	LC	7	1,01
		<i>Leptopelis sp</i>		31	4,48
		<i>Leptopelis millsoni</i> (Boulenger, 1895)	LC	1	0,14
Rhacophoridae Hoffman, 1932 (1858)	Chiromantis Peters, 1854	<i>Chiromantis rufescens</i> Günther, 1869	LC	2	0,29
Hemisotidae Cope, 1867	Hemisis Günther, 1859	<i>Hemisis guineensis</i> Cope, 1865	LC	9	1,30
		<i>Hemisis marmoratus</i> (Peters, 1854)	LC	2	0,29
		<i>Hemisis olivaceus</i> Laurent, 1963	LC	23	3,32
Dicroglossidae Anderson, 1871	Hoplobatrachus Peters, 1863	<i>Hoplobatrachus occipitalis</i> (Günther, 1858)	LC	123	17,77
	Amnirana Tchudi, 1838	<i>Amnirana galamensis</i> (Duméril & Bibron, 1841)	LC	3	0,43
Ranidae Batsch, 1796		<i>Amnirana amnicola</i> (Perret, 1977)	LC	1	0,14
		<i>Amnirana darlingi</i> (Boulenger, 1902)	LC	1	0,14
		<i>Amnirana albolabris</i> (Hallowell, 1856)	LC	169	24,42
Phrynobatrachidae Laurent, 1941	Phrynobatrachus Günther, 1862	<i>Phrynobatrachus natalensis</i> (Smith, 1849)	LC	1	0,14
		<i>Phrynobatrachus auritus</i> Boulenger, 1900	LC	2	0,29
	Ptychadena Boulenger, 1917	<i>Ptychadena bibroni</i> (Hallowell, 1845)	LC	3	0,43
		<i>Ptychadena christyi</i> (Boulenger, 1919)	DD	1	0,14
Ptychadenidae Dubois, 1987		<i>Ptychadena ingeri</i> Perret, 1991	DD	2	0,29
		<i>Ptychadena mascareniensis</i> (Duméril & Bibron, 1841)	LC	13	1,88
		<i>Ptychadena sp</i>		1	0,14
	Sclerophrys Tschudi, 1838	<i>Sclerophrys regularis</i> (Reuss, 1833)	LC	1	0,14
		<i>Sclerophrys camerunensis</i> (Parker, 1936)	LC	1	0,14
		<i>Sclerophrys pusilla</i> (Merten's 1937)		2	0,29
Bufonidae Gray, 1825		<i>Sclerophrys tuberosa</i> (Günther, 1858)	LC	11	1,59
		<i>Sclerophrys latifrons</i> (Boulenger, 1900)	LC	3	0,43
		<i>Sclerophrys gracilipes</i> (Boulenger, 1899)	LC	1	0,14
		<i>Sclerophrys sp</i>		26	3,76
	Xenopus Wagler, 1827	<i>Xenopus pygmaeus</i> Loumont, 1986	LC	2	0,29
		<i>Xenopus sp</i>		5	0,72
Pipidae Gray, 1825	Hymenochirus	<i>Hymenochirus boettgeri</i> (Tornier, 1897)	LC	2	0,29
		<i>Hymenochirus sp</i>	LC	24	3,47
		<i>Hymenochirus boulengeri</i> Witte, 1930	DD	34	4,91
		<i>Hymenochirus sp nov</i>		3	0,43
11	17	53		692	100

The Table 1 shows that 692 specimens were caught in the RFO. These specimens were divided into 53 species, 17 genera and 11 families. All the inventoried species belong to the Order of the Anurans. The different families of amphibians collected are mainly represented by Hyperoliidae (159 specimens: 22.97%), Pyxicephalidae (8 specimens (1.15%)), Arthroleptidae (54 individuals: 7.80%), Rhacophoridae (2 specimens: 0.28%), Hemisotidae (34 specimens: 4.91%), Dicroglossidae (123 specimens: 17.77%), Ranidae (174 individuals: 25.14%), Phrynobatrachidae (3 individuals: 0.43%), Ptychadenidae (22 specimens: 3.17%), Bufonidae (45 specimens: 6.5%) and Pipidae (68 individuals: 9.82%).

The Ranidae family is represented by 1 genus and 6 species. This family was the most abundant with 174 specimens (25.14%), followed by Hyperoliidae (159 individuals: 22.97%). All species caught in this family are in least concern (LC) on the IUCN Red List. The Dicroglossidae family is represented by 123 specimens (17.77%). This family is currently represented by a genus and species in the DRC, *Hoplobatrachus occipitalis*. The Rhacophoridae family is represented by 2 specimens (0.28%) and the Phrynobatrachidae are represented by 3 specimens (0.43%). The Hyperoliidae family is diversified into 5 genera, namely: *Afrixalus*, *Cryptothylax*, *Hyperolius*, *Kassina* and *Phlyctimantis*. The genera *Afrixalus*, *Kassina*

and *Cryptothylax* had only been represented by one species each. All inventoried species of the Hyperoliidae family have minor concerns (LC) on the Red List, except *Hyperolius cf. brachiofasciatus* and *Hyperolius ferrugineus* which are listed under insufficient data (DD). In addition, the family of Pyxicephalidae is represented by 8 specimens diversified into 2 species and grouped into 1 genus. These species are: *Amietia chapini* (1 specimen: 0.14%) and *Amietia angolensis* (7 specimens: 1.01%). The Arthroleptidae family is represented by 2 genera: *Arthroleptis* and *Leptopelis*.

The first is represented by a single species: *Arthroleptis sp* (3 specimens out of 692: 0.43%) and the second is diversified into 5 species: *Leptopelis christyi* (3 specimens: 0.43%), *Leptopelis ocellatus* (7 specimens: 1.01%), *Leptopelis notatus* (2 individuals: 0.29%), *Leptopelis calcaratus* (7 individuals: 1.01%), *Leptopelis sp* (31 specimens: 4.48%) and *Leptopelis millsoni* (1 specimen: 0.14%). All species in this family are of minor importance (LC) on the IUCN Red List.

The Rhacophoridae family is represented by 1 genus and 1 species: *Chirromantis rufescens*: 2 specimens (0.29%) (LC). The Hemisotidae family is diversified into 3 species: *Hemisus guineensis* (9 specimens: 1.3%), *Hemisus sp* (2 individuals: 0.29%) and *Hemisus olivaceus* (23 individuals: 3.32%). However, the family of Phrynobatrachidae is represented by 3 specimens and 2 species: *Phrynobatrachus natalensis* (1 individual: 0.14%) and *Phrynobatrachus auritus* (2 individuals or a proportion of 0.29%). All harvested species are of minor importance (LC).



**Figure 2.** Presentation of some amphibian species captured in RFO. Left to right: *Sclerophrys camerunensis* and *Sclerophrys sp*.

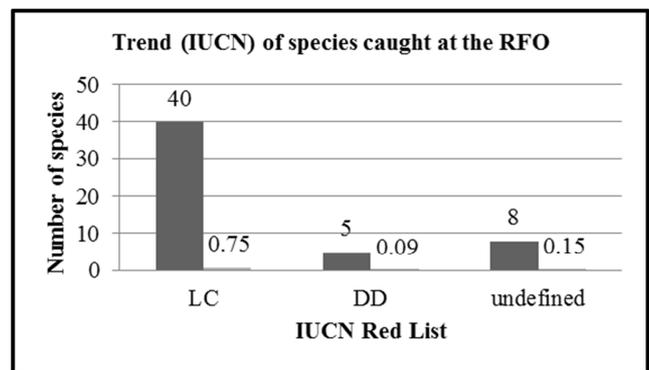
The family of Ptychadenidae includes 22 specimens divided into 7 species. The different species collected are: *Ptychadena bibrioni* (3 specimens: 0.43%), *Ptychadena christyi* (1 specimen: 0.14%), *Ptychadena ingeri* (2 individuals: 0.29%), *Ptychadena mascareniensis*: 13 individuals: 1.88%) and *Ptychadena sp* are each represented with 1 specimen (0.14%). Among these species of the family Ptychadenidae; *Ptychadena christyi* and *Ptychadena ingeri* (DD) are on the IUCN Red List. The Bufonidae family is represented by 1 genus and 7 species: *Sclerophrys regularis* (1 specimen: 0.14%), *Sclerophrys camerunensis*: 0.14%, *Sclerophrys pusilla* (2 individuals: 0.29%), *Sclerophrys tuberosa* (11 specimens: 1.59%), *Sclerophrys latifrons* (3 specimens: 0.43%), *Sclerophrys gracilipes* (1 specimen: 0.14%) and *Sclerophrys sp*: 26 individuals (3.76%). All species in this family for this research are (LC). But,

individuals belonging to the Pipidae family are diversified into 2 genera and diversified into 6 species: *Xenopus pygmaeus*: 2 individuals (0.29%), *Xenopus sp*: 5 specimens (0.72%). The genus *Hymenochirus* is diversified into 4 species: *Hymenochirus boettgeri* (2 specimens or 0.29%), *Hymenochirus sp* (23 specimens: 3.32%), *Hymenochirus boulengeri* (33 individuals: 4.77%) and a *Hymenochirus sp* in studie (3 individuals: 0.43%). *Hymenochirus boulengeri* is (DD).

Our descriptive analysis of the data shows that the mean was from the sample was 13.06 with a standard error of 4.065. The median was 3 while the mode was 1, the standard deviation was 29.59 and the variance of the sample (875.59). The minimum was 1 and the maximum 169. The sum of the sample was 692; the asymmetry coefficient 4.037 and the number of species were 53 for a confidence level (95.0%) was 8.16.

### 3.2. Conservation Status of Amphibian Species Caught in the RFO

The following figure shows that we caught 53 species of amphibians in the Okapi Wildlife Reserve. We didn't catch endangered species. The majority of species (40 out of 53 species in total, or 75.47%) are of minor concern (LC), 8 species or 15.09% undefined species and 5 species (9.43%) are species with insufficient data.



**Figure 3.** Trend (IUCN) of species caught at the RFO.

## 4. Discussion

Herpetological studies in DRC's protected areas are rare, although the DRC is a global challenge both in terms of biodiversity and the fight against climate change [13]. But protected areas face illegal hunting with traditional and automatic weapons, non-organic agriculture, population explosion and weak legislation.

This rapid inventory at the RFO provided 53 species (Table 1) of amphibians. The specimens collected for this mission were georeferenced and put online via the specialized online database (GBIF.org). This result provides sufficient evidence that the RFO region is more diverse in amphibians than Gabon, where only 104 species are found [16] although the taxonomic list is based on a geo-referenced database of 4,200 data collected from data published by several authors and about 16

amphibian species in Namibia [17]. Gabon's national parks-although preliminary results for five of the 13 parks: Crystal, Ivindo, Loango, Lopé and Moukalaba-Doudou, indicate (86%) of the 88 known species [18].

As for the conservation and threat status of amphibians in the RFO, more detailed studies must be carried out to determine the status of each amphibian species. Our research showed that 53 species of amphibians caught were collected and the majority (75.47%) had a minor concern (LC) but 15.09% represent species whose threat status is not defined on the IUCN red list; a real dark area on this taxon because the species *Hymenochirus sp* would be very interesting and is under review while the genus could be (DD) and, the results will be provided later and; 9.43% are species with insufficient data (DD). This proves that amphibians in the Okapi Wildlife Reserve are less well known. These preliminary results are our main hypothesis that amphibians are rich and diverse in the Okapi Wildlife Reserve, but they are not well studied. Concerning the field capture sessions, we agree with [18] that rainy evenings were the most favourable for amphibian capture, as they were manifested by a large concert.

## 5. Conclusion

In this research, 692 amphibian specimens were caught in the Okapi Wildlife Reserve and divided into 53 species, 17 genera and 11 families. All the inventoried species belong to the Order of the Anurans. These results have shown that the amphibian fauna of the Okapi Wildlife Reserve is rich and diverse. This is why it must be a priority in the conservation plan, because protecting amphibians also means protecting wetlands and their biodiversity. Of all these known families, this research identified 11 out of 15 families (73.33%), with the exception of Brevicipitidae and Caeciliidae; 2 out of 15 families (13.33%).

We insist that further long-term research must cover the entire RFO so that the full list of the Reserve is known exactly.

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